SULFUR FERTILIZATION AND NUTRIENT DISTRIBUTION IN A FLORIDA SPODOSOL PROFILE UNDER WHITE CLOVER-PENSACOLA BAHIAGRASS

BY

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Dedicated to

my parents Joao and Margarida,

my wife Marisa,

and my daughter Juliana.

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TABLE OF CONTENTS

	PAGE
ACKNOWLEDGMENTS	iii
ABSTRACT	vii
CHAPTER I	
INTRODUCTION	1
CHAPTER II	
LITERATURE REVIEW	4
Characteristics of the Studied Soil and Forages	4
Soil	4
Forages	5
Sulfur and the Environment	7
Sulfur in Soils	8
Forms of Sulfur in Soils	8
Behaviour of Sulfur in Sandy Soils	11
Assessing Sulfur Status in Soils	13
Sulfur in Plants	17
Uptake and Functions of Sulfur in Plants	17
Sulfur Relations to Other Plant Nutrients	18
Assessing Sulfur Status in Plants	19
Forage Responses to Sulfur	21
Clovers Grown Alone	22
Clover-Grass Mixed Systems	32
Nutrient Distribution in the Soil Profile	40
CHAPTER III	
RESPONSES TO SULFUR FERTILIZATION BY A WHITE CLOVER-PENSACOLA BAHIAGRASS SWARD	45
Introduction	45
Materials and Methods	47
Experiment No. 1	47
Experiment No. 2	50
Experiment No. 3	52
Results and Discussion	53
Experiment No. 1	53
Experiment No. 2	62
Experiment No. 3	65
Summary	68

CHAPTER IV
EFFECTS OF SULFUR AND MOLYBDENUM APPLICATIONS ON WHITE CLOVER ALONE AND MIXED WITH PENSACOLA BAHIAGRASS..... 71
Introduction..... 71
Materials and Methods..... 72
Greenhouse Experiment..... 72
Field Experiment..... 74
Plant and Soil Analyses..... 75
Statistical Analysis..... 76
Results and Discussion..... 76
Greenhouse Experiment..... 76
Field Experiment..... 86
Summary..... 91

CHAPTER V
EFFECTS OF TWO SOURCES OF SULFUR COMBINED WITH POTASSIUM ON WHITE CLOVER GROWN IN A GREENHOUSE..... 93
Introduction..... 93
Materials and Methods..... 94
Results and Discussion..... 96
Summary..... 116

CHAPTER VI
CHANGES AND DISTRIBUTION OF EXTRACTABLE NUTRIENTS, pH, AND ORGANIC CARBON IN A SPODOSOL PROFILE UNDER A LONG-TERM DIFFERENTIALLY LIMED AND FERTILIZED LEGUME-GRASS SWARD..... 118
Introduction..... 118
Materials and Methods..... 120
Results and Discussion..... 123
Summary..... 149

CHAPTER VII
CONCLUSIONS..... 151

APPENDIX
NUMERICAL DATA ON CHANGES AND DISTRIBUTION OF EXTRACTABLE NUTRIENTS, pH, AND ORGANIC CARBON IN A SPODOSOL PROFILE..... 157

REFERENCES..... 165

BIOGRAPHICAL SKETCH..... 181

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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BY

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A Spodosol that has supported white clover (Trifolium repens L.)-Pensacola bahiagrass (Paspalum notatum Flugge) swards since 1952 was used in a series of greenhouse and field experiments to test S fertilization with and without Mo and K fertilization, and to study nutrient distribution in the soil profile.

In the greenhouse, white clover development depended on S fertilization. Sulfur application increased yield, and N and S concentrations and contents, but decreased N:S ratio, and K and Mo concentrations in the legume. Previous S fertilization in the field did not affect white clover growth. Sulfur-deficient plants had a reduced number of flower heads and nodules, delayed flowering, reduced tillering, smaller nodules, and reddening of petioles and

stolons. Molybdenum fertilization increased Mo concentration and content in the legume. Yield and K concentration in the legume were increased by high K application. Current S application decreased soil pH and extractable soil K, but increased total and extractable soil S.

In the field, S fertilization increased yield, and N and S contents in forages every year. Except when damaged by severe frost, white clover had increased S concentration and reduced N:S ratio due to current S fertilization. Molybdenum concentration in the legume was decreased by S fertilization. Stolon-root tissues of bahiagrass did not store S during the spring. The deep root system of bahiagrass seemed to take up S present in deeper soil layers. Extractable soil K, and K concentrations in white clover tissue and stolon-root tissue of bahiagrass were lower from the S-fertilized plots. Leaching of S was considered most responsible for low apparent S recovery from the fertilizer.

Several measurements were done for soil samples collected at nine soil depths in the mixed sward amended and fertilized for 32 years. Limed plots, which contained white clover, had higher total N in the top 7.5 cm of soil than did unlimed plots. Most of the measured variables decreased with increasing soil depth, and the major changes occurred in the top 30 to 45 cm of soil. Extractable S was highest at 75 to 90 cm, and S fertilization lowered extractable K throughout the soil profile.

CHAPTER I
INTRODUCTION

Permanent pastures composed of grasses and legumes have recently received special attention throughout the world. The inclusion of legumes in the pasture has many advantages. They provide N for the system, increase the amount of protein available for the grazing animal, and extend the grazing period during the year.

There are about 6 million hectares of Spodosols in Florida. Much of this soil order is located in the North Central part of the state, either in use as, or potentially available for, pastures (Carlisle and Brown, 1982). Clover-grass mixtures have been shown to be economical in beef cow-calf operations in that area (Koger et al., 1961, 1977).

One of the major concerns in a permanent pasture is to keep a very productive stand for a long period, without having to resort to frequent renovation, which is an expensive operation. An adequate pasture fertilization program is necessary to provide sufficient plant nutrients for high forage productivity. Adequate soil nutrient status is particularly important for the establishment and maintenance of legumes in any pasture. Both the functioning of the N_2-fixing system and the development and growth of the

plants depend on an adequate supply of the essential elements.

A white clover (Trifolium repens L.)-Pensacola bahiagrass (Paspalum notatum Flugge) mixed sward was established in an experimental area of the University of Florida on a Spodosol in 1952, and has been managed under periodic liming and fertilization practices. Nitrogen, an essential nutrient for improving productivity of any established pasture, had been supplied entirely through the N_2-fixing system of the legume. However, the biological supply of N to the sward has recently been reduced because white clover has not grown as well in the last several years as it had previously. A nutritional limitation for adequate performance of white clover in the area was suggested.

Through the frequent use of dolomitic lime and high analysis fertilizers (as was done in the experimental area) and the existence of legumes, inputs of N, P, K, Ca and Mg have been made to the system, but the same is not true for S.

Sulfur deficiency for crops and forage plants has been reported throughout the world. Although legumes have exhibited higher S requirement than grasses, both plant families have shown better performance in pastures receiving S applications.

There is scarce information on the needs and effects of S fertilization, as well as the nutrient distribution in

various depths of a soil profile, on such a long-term mixed system.

A series of experiments was devised to study the effects of S additions for the mixed sward in the field and for white clover grown in pots, and to observe how the nutrients are distributed in the soil profile under the long-term sward.

CHAPTER II
LITERATURE REVIEW

Characteristics of the Studied Soil and Forages

Soil

The Myakka series (sandy, siliceous, hyperthermic Aeric Haplaquod) represents the poorly drained Spodosols (commonly known as flatwoods) formed on deep sandy marine deposits on level relief. The typical pedon of such soils has A, E, Bh, BC, and C horizons (Carlisle and Brown, 1982).

Sand is the predominating particle-size (usually more than 90%), while the clay fraction constitutes up to 5% throughout the profile of Spodosols (Buol et al., 1980). The clay fraction of Myakka horizons commonly has 89 to 99% quartz as the dominant mineral (Mitchell, 1980).

The water table in these soils, in most years, is less than 25 cm below the soil surface for 1 to 4 months, and when located in low lands, they may have standing water for 6 to 9 months (Carlisle and Brown, 1982).

Under natural conditions, Myakka soils are strongly acidic and have very low supplies of plant nutrients (Bryan, 1958), but can be quite productive if properly limed and fertilized (Blue and Carlisle, 1985). The native vegetation

on the Myakka soils includes longleaf and slash pine trees (Pinus spp.), with saw-palmetto (Serenoa repens Bartr. Small), runner oak (Quercus minima Sarg.), gallberry (Ilex glabra L.), and scattered fetterbushes (Pieris nitida Benth & Hook) as understory plants (Carlisle and Brown, 1982).

When moisture is controlled, these soils can be used for forestry, pasture, hay land, and cultivated crops such as vegetables, berries and silage crops (Buol et al., 1980).

Forages

A legume, white clover (Trifolium repens L.), and a grass, Pensacola bahiagrass (Paspalum notatum Flugge), were the forage species used in the present studies.

White clover is found throughout the temperate regions of the world, in subtropical regions such as the Gulf Coast of the United States, and in high altitudes near the tropics (Leffel and Gibson, 1973). Although it is a perennial plant in temperate areas, white clover behaves much as an annual, winter grown forage in Florida (Baltensperger et al., 1984).

This legume has a prostate growth habit. Stolons arising from the primary stem develop radially. A single plant might occupy an area of a square meter or more. Each compound leaf normally contain three leaflets, and each plant develops a primary taproot from which a series of secondary roots originates (Leffel and Gibson,1973).

White clover is considered the best cool-season legume for fertilized Spodosols and other high-moisture

supplying soils (Prine et al., 1984). Osceola, a new white clover cultivar, was developed by members of the Agronomy Department, University of Florida; this cultivar has compared favorably to other cultivars, such as Louisiana, Nolin's, Regal, and Tillman in yield, growth period, seed production, and N concentration in the plants. Its average annual yields have been 5500 to 8500 kg ha^{-1} in several Florida locations (Baltensperger et al., 1984; Prine et al.,1984).

Grasses of the genus Paspalum are important in subtropical agriculture, and are particularly well adapted to much of the southeastern United States. Among the economically important species of Paspalum, bahiagrass is the most widely grown. An estimate of one million ha of Pensacola bahiagrass pastures exist in Florida, and are used primarily for beef cattle. Pensacola bahiagrass has longer, narrower leaves, taller seed stalks, higher seed production, higher forage yield, and higher nutritive value than Common, Argentine, Paraguay, and Wilmington bahiagrass cultivars. In several Florida locations and under various fertilization programs, it has produced average annual oven-dry forage yields of 2200 (low NPK) to 14500 (high NPK) kg ha^{-1}, and animal gains of 170 to 850 kg ha^{-1} (Chambliss and Jones, 1981; Ward and Watson, 1973).

Pensacola bahiagrass is well adapted to poorly drained and low-fertility soils, has woody horizontal rhizomes, and a fibrous root system capable of growing to a depth of 2 m

or more. It has some cold tolerance, but its yield is low during the winter months. The top may be killed by moderate frosts. In North Florida, it is productive in spring, summer, and mid-fall (Chambliss and Jones, 1981; Ward and Watson, 1973).

Sulfur and the Environment

Sulfur occurs in nature in solid, liquid, and gaseous phases. It is the thirteenth most abundant element in the earth's crust, and its content usually ranges between 600 and 1000 mg kg^{-1}. Sulfate salts in sea water reach a concentration of about 900 mg S L^{-1}, and constitute a very large reservoir of S (Atlas and Bartha, 1981; Goldberg, 1963; Tisdale et al., 1985).

The amount of atmospheric S is dependent on the contribution from several sources. Common sources are: SO_2 emissions from industrial activities, residential burning of fossil fuels and plant materials, volatilization of sulfides during biological decay, reduction of sulfates in waterlogged soils, volcanic emissions, and sea spray (Fox et al., 1983). Once in the atmosphere, SO_2 can be directly utilized by plants, can cause severe damage to the vegetation (if present in high concentrations), or can be returned to the soil in rain (Epstein, 1972; Fox et al., 1983; Lauenroth and Preston, 1984). Although the man-made contributions to atmospheric S are of much less magnitude than those resulting from microbiological activities, they have been the

focus of the attention on environmental problems in the last decade (Fox et al., 1983; Tisdale et al., 1985).

The amount of S returned to agricultural land through rainfall each year is highly variable among sites (Fox et al., 1983). In the vicinity of Gainesville, Florida, it has been about 4 to 8 kg ha^{-1} in recent years, with about two-thirds of the S deposition occurring during the summer months (Brezonik et al., 1980).

Sulfur is an essential element for living organisms, and it is assimilated by plants, animals, and microorganisms into the S-containing amino acids (cystine, cysteine, and methionine). These categories of organisms play significant roles in S cycling in grassland systems. Microorganisms perform most of the S transformations in soils, and in the rumen of ruminants. Forage plants absorb mainly inorganic S, which is primarily assimilated into organic compounds subsequently eaten by animals. A part of the S taken by the animals is returned to the pasture system as excreta, whereas another portion is incorporated into the body and later exported from that system (Kamprath and Till, 1983; Siebert and Vijchulata, 1983).

Sulfur in Soils

Forms of Sulfur in Soils

Sulfur occurs in soils in both organic and inorganic forms. In soils of humid regions of the world more than 90% of the total S occurs in organic combinations (Stevenson,

1982). Despite this fact, the chemical nature of the organic forms is not as well defined as it is for inorganic forms (Tabatabai, 1982).

Organic S compounds in soils are usually separated into two groups according to the bonding between S and C (Kamprath and Till, 1983; Stevenson, 1982; Tabatabai, 1982; Tisdale et al., 1985). One group has S directly bonded to C, and the other not directly bonded. Each fraction constitutes about 50% of the organic S in soils (Bettany et al., 1973; Freney, 1967; Tabatabai and Bremner, 1972). The non-C-bonded organic S can be reduced to H_2S by hydriodic acid (Freney, 1961). It might occur in soils as ester sulfates, such as phenolic sulfates, choline sulfate, and sulfate esters of carbohydrates and lipids (Freney and Stevenson, 1966). Part of the C-bonded S fraction may be reduced to H_2S by Raney Ni in alkaline medium (Lowe and De Long, 1963), and includes the S-containing amino acids methionine, cystine, and cysteine (Freney and Stevenson, 1966). That part of C-bonded S which is not reduced by Raney Ni is an unidentified fraction considered as residual or inert S (Tisdale et al., 1985). The vitamins thiamine and biotin; some antibiotics, (e.g., penicillin, and gliotoxin); glutathione; and coenzyme A are C-bonded S compounds which are expected to occur in trace amounts in soils (Stevenson, 1982).

Although plants can take up S from the soil organic fraction (Bardsley, 1960; Freney et al., 1975), the frac-

tionation of organic S in soils is of low value for predicting S requirements for crops (Probert and Samosir, 1983).

The inorganic forms of S in soils include readily-soluble sulfate, adsorbed sulfate, precipitated sulfates, and reduced S compounds (Probert and Samosir, 1983; Tabatabai, 1982; Tisdale et al., 1985). In well-drained and well-aerated soils, S is mainly present in the sulfate form, whereas reduced S compounds predominate under anaerobic conditions (Tabatabai, 1982).

The amount of readily-soluble sulfate is commonly very low in the surface of well-drained soils (Tabatabai, 1982), and S-deficient sandy soils usually have less than 5 mg S kg^{-1} of soil in this form (Tisdale et al., 1985).

Sulfate, like some other anions, e.g., phosphate and molybdate, can be adsorbed on soil colloids. This anion typically undergoes specific adsorption on mineral surfaces (Hingston et al., 1972). The adsorption occurs where 0 atoms are partly coordinated on surfaces of Al and Fe hydrous oxides, and on broken edges of the layer silicates (Gast, 1977). Therefore, the amount of sulfate adsorbed in any soil depends on the amount and distribution of those surfaces in the soil profile.

Precipitated or relatively insoluble forms of S occur in soils as Ba and Sr sulfates, Fe and Al sulfates (e.g., jarosite and coquinbite), and sulfate coprecipitated with $CaCO_3$ (Tabatabai, 1982).

In O_2-deficient, flooded soils, reduced forms of S commonly occur and sulfide is the main stable form. The reduction of sulfate to sulfide is carried out by bacteria, and the sulfide produced is extremely toxic to plant roots and soil organisms (Alexander, 1977).

In terms of contributions to meet plant needs for S, the readily-soluble and adsorbed sulfate are considered to be the major portions of the inorganic S in the soil (Kamprath and Till, 1983).

Behaviour of Sulfur in Sandy Soils

Sulfur in soils is subjected to mineralization-immobilization turnover. In this process, sulfate is either released from the soil organic fraction, including plant and animal residues, or is incorporated into the organic S pool in the soil (Tisdale et al., 1985). This continuous process is a result of the microbial activity, which is influenced by soil factors such as moisture, temperature, availability of mineral nutrients, pH, and O_2 supply (Alexander, 1977).

Soil C:S and N:S ratios and the presence or absence of plants, have been suggested as decisive factors to define which direction of the turnover will predominate (Anderson, 1975; Freney and Spencer, 1960; Stewart, 1969). When net mineralization occurs, usually it represents only 1 to 2% of the organic S in the soil. This quantity rarely will supply an adequate amount of S for optimum growth of the plants (Kamprath and Till, 1983).

Readily-soluble sulfate usually undergoes a downward movement in the soil profile, in a process whose intensity is directly related to the amount of percolating water (Chao et al., 1962).

Sulfate applied as fertilizer to soils may be leached from the soil (Neller, 1958; Shock et al., 1983; Watson, 1969). Up to 78% of the S applied to lysimeters has been found in leachates during one growing season (McKell and Williams, 1960). Since the Myakka soils have rapid permeability in the A and E horizons, and moderate or moderately rapid permeability in the Bh horizon, it is expected that sulfate-S would be easily leached at least from the surface horizons.

Adsorption of sulfate in the soil is influenced by several soil properties. Commonly, sulfate adsorption is greater under conditions of high clay content, predominance of kaolinitic minerals, presence of hydroxides of Fe and Al, strong soil acidity, low amounts of phosphate and molybdate in the soil, and high organic matter content (Tisdale et al., 1985; Whitehead, 1964). Appreciable amounts of sulfate are adsorbed especially in the subsoil of Ultisols and Oxisols, and this sulfate is considered to be available for plants (Kamprath and Till, 1983). In an amended and fertilized Spodosol, it is possible to estimate that adsorbed sulfate probably may not contribute substantially to the inorganic S pool in the soil.

Anaerobic soil conditions favor the conversion of inorganic sulfate to sulfide. The process results in a smaller amount of sulfate available for plant use and, at the same time, increases the amount of H_2S, which may be toxic to plants. This might occur in Aquods (Spodosols commonly saturated with water), and this process would have some effect on the transformation of inorganic sulfate.

Assessing Sulfur Status in Soils

As previously discussed, S occurs in soils both in organic and inorganic forms. There are a number of direct methods for determination of total and inorganic S, but no satisfactory direct method for determining the total organic S has been described (Tabatabai, 1982). A detailed description of the existing methods for S determination was compiled by Beaton et al. (1968).

Procedures for S determinations have been tedious. Attempts to develop new methods for S or to improve the existing ones have been focused on the combination of accuracy, precision, and speed (Tabatabai and Bremner, 1970a).

In agricultural studies, total soil S and extractable SO_4 are usually emphasized (Randall and Sakai, 1983). Determination of total S is usually carried out by oxidation of all S forms to sulfate. In some methods, sulfate is reduced to sulfides. Among the oxidation-based methods, fusion of the sample with some salts, wet digestion with acid or

alkali, dry ashing with bicarbonate-oxide mixture, X-ray fluorescence spectrometry, and direct combustion of the sample in a high-frequency induction furnace have been extensively used (Beaton et al., 1968; Brown and Kanaris-Sotiriou, 1969; Tabatabai, 1982).

In the high-frequency induction furnace, the soil sample is burned at a high temperature (1400 to 1500°C) in a stream of O_2, so that all S forms are converted to SO_2; complete conversion is only achieved at such high temperature (Thompson and Allbright, 1970). The gases evolved are filtered and analyzed for SO_2 according to previous calibration of the analyzer with known standards.

Tabatabai and Bremner (1970b) compared total S determinations in soils by the methods of wet alkaline oxidation, wet acid oxidation, dry ashing, X-ray fluorescence and direct combustion in induction furnace. In the direct combustion method, they collected SO_2 gases in diluted HCl and titrated the solution with a KI solution. They reported that the direct combustion method gave 3% higher average values for total S than did the wet oxidation methods. Also, the coefficient of variation for the samples analyzed in the induction furnace was about 6 to 7%, while for the wet digestion they ranged from 1 to 2%.

Hern (1984) added vanadium pentoxide to the samples before burning, and used an infrared detection cell to determine S by direct combustion in an induction furnace.

He reported improvements in the accuracy and precision of the method, as a consequence of those modifications.

The use of a direct combustion method with an infrared detector for determining total S in an Ultisol ranging from 27 to 76 mg S kg^{-1} of soil was made by Hue et al. (1984).

Determination of the soil concentration of S that might be available for plant uptake can be done by several methods. Usually the methods involve extraction of inorganic sulfate, extraction of both inorganic sulfate and portions of organic fraction, mineralization of S during incubation, and biological assays (Beaton et al., 1968; Reisenauer et al., 1973).

Chemical methods for evaluating S availability in the soil usually are based on soil extraction with water (Freney, 1958), Morgan's solution - sodium acetate and acetic acid - (Chesnin and Yien, 1950), neutral ammonium acetate (McClung et al., 1959), sodium bicarbonate (Bardsley and Kilmer, 1963), calcium chloride (Williams and Steinbergs, 1959), lithium chloride (Arkley, 1961), and phosphate solutions (Fox et al., 1964). Among those extractants, 0.15% $CaCl_2$, 0.1 M LiCl, and 500 mg P L^{-1} as $Ca(H_2PO_4)_2$ have been preferred because they are good extractants for SO_4, and also prevent extraction of non-available S (Barrow, 1967; Beaton et al. 1968; Tabatabai, 1982). The phosphate solution also has the advantage of displacing the adsorbed fraction of inorganic soil sulfate (Barrow, 1967).

Once extracted from the soil, sulfate is determined by gravimetric, colorimetric, turbidimetric, nefelometric, titrimetric, ion-chromatographic or X-ray fluorescence techniques (Beaton et al., 1968). The gravimetric method is the most traditional (Blumenthal and Guernsey, 1915), the methylene blue colorimetric method coupled with the reduction of sulfate to sulfide (Johnson and Nishita, 1952) is suggested as very precise and accurate (Tabatabai, 1982), and the turbidimetric method of Chesnin and Yien (1950) is the most widely used (Reisenauer et al., 1973).

In the turbidimetric method, $BaSO_4$ formed after the addition of $BaCl_2$ to the extract is suspended in the liquid and the suspension is stabilized by the addition of a stabilizer such as gum arabic. The addition of a dilute seed suspension of $BaSO_4$ improved the original method, and is considered especially important with samples containing small amounts of sulfate (Massuomi and Cornfield, 1963).

Very high correlations between plant S uptake and extractable soil sulfate have been reported (Bardsley and Lancaster, 1960; Barrow, 1967; Fox et al., 1964; Hue et al., 1984; Mitchell, 1980; Rehm and Caldwell, 1968; Williams and Steinbergs, 1959). Also, a range of 10 to 12 mg S kg^{-1} of soil has been suggested as a critical level of extractable sulfate from the soil for temperate forage legumes (Dawson, 1969; Fox et al., 1964; Grava, 1971; Harward et al., 1962; Walker and Doornenbal, 1972).

Sulfur in Plants

Uptake and Functions of Sulfur in Plants

Sulfur is absorbed by plants mainly in the form of sulfate ions (Epstein, 1972). Other forms of S taken up by plants are sulfide and organic compounds such as cysteine, and methionine (Bardsley, 1960; Freney et al., 1975; Salisbury and Ross, 1978).

The movement of sulfate to the root surface is considered to occur predominantly by mass-flow (Barber, 1962), and the uptake of the anion into roots is mainly a dual pattern metabolic process (Epstein, 1972). Sulfate is then actively transported through the plasmalemma and tonoplast of root cells on its way to xylem, which is responsible for upward transport of nutrients (Cram, 1983; Epstein, 1972).

Sulfate in the plants undergoes assimilatory reduction in order to supply sulfide for the synthesis of organic sulfur compounds (Thompson, 1967). The series of reactions for sulfate reduction takes place mainly in the leaves. The reaction rate can be lower than the uptake, leading to sulfur accumulation in the plant when the supply is ample (Rennenberg, 1984). In clover plants, sulfate accumulates more in the stems than in the petioles or leaflets, when S supply is high (Jones, 1962).

Sulfur-containing organic compounds in plants include the amino acids cystine, cysteine, methionine, and proteins containing them, vitamins (e.g., thiamine, biotin), coenzyme

A, ferredoxins, and mercaptans in some species. The enzyme complex called nitrogenase, essential for the N_2-fixation process, is known to contain S (Epstein, 1972).

Good S supply to legumes has resulted in beneficial effects on the N_2-fixing system in terms of number and development of nodules, and the amount of N_2 fixed (Adams, 1973; Anderson and Spencer, 1950; Gates, 1974; Nuttal, 1985; Shock et al., 1983; Shock et al., 1984b). However, Robson (1978) suggested that S was not specifically involved in N_2-fixation, and that an S deficiency would affect N metabolism rather than N_2 fixation.

Sulfur Relations to Other Plant Nutrients

The mutual effects of S and other nutrients have been noted. As indicated by the law of minimum, any deficiency of a single nutrient would limit S utilization by plants (Epstein, 1972). However, special attention has been given to N and P. Jordan et al. (1961) and Robinson and Jones (1972) reported that forage legumes had a better growth and higher S uptake in response to highly available S only when P availability was adequate.

An adequate amount of N should be supplied for the non-leguminous plants to improve growth, and to make better utilization of the absorbed S in the metabolic processes (Anderson and Spencer, 1950, Daigger and Fox, 1971, Spencer and Freney, 1980; Terman et al., 1973). Dijkshoorn et al.

(1960) found a definite stoechiometric relationship between S and N in the plant.

The negative effect of adequate S supply to plants on Mo concentrations is widely known. Stout et al. (1951) first reported that adequate sulfate in a nutrient medium depressed Mo absorption by the plants. Later, this antagonistic relationship was also reported by Gupta and Munro (1969), Singh and Kumar (1979), Reddy et al. (1981), and Reisenauer (1963). The competition between SO_4^{2-} and MoO_4^{2-} was suggested to occur at the absorption sites of the roots, and to be due to similar size and charge of the two anions.

Assessing Sulfur Status in Plants

The S adequacy in plants has been determinated mainly from the total S and/or sulfate-S analysis and N:S ratio in the plant tissue (Metson, 1973). Procedures for ashing of plant samples, S extraction, and analytical techniques were reviewed in detail by Beaton et al. (1968).

To determine total S in plant tissues, dry ashing in an O_2-enriched environment or by ignition or combustion, and wet ashing with a mixture of acids have been frequently used. After being converted to sulfate or sulfide, S is analyzed by gravimetric, turbidimetric, colorimetric, or iodimetric techniques (Beaton et al., 1968).

Critical concentrations of total S in the forage grasses and clovers usually fall in ranges of 1.0 to 1.5 and 1.5 to 2.1 g kg^{-1}, respectively (Metson, 1973).

The direct combustion of samples in a stream of O_2 in an induction furnace at a high temperature has been one of the chosen methods recently for determination of total S in plant tissues (Hern, 1984; Hue et al., 1984; Mitchell, 1980). Details of the method are the same as for soil analysis.

Based on the observation that plants having any S in excess to that necessary for the metabolism would accumulate the element in the sulfate form, Jones (1962) proposed the use of sulfate-S determination as a diagnostic tool for S deficiency in forage legumes. He suggested a value of 170 mg SO_4-S kg^{-1} of plant tissue as a critical level for Trifolium subterraneum. Sulfate-S has been used as a diagnostic parameter for grasses and legumes, and the critical level has ranged between 100 and 400 mg kg^{-1} plant material (Metson, 1973).

Dijkshoorn et al. (1960) devised a method of diagnosing the S status in plants by the use of the near-constant N:S ratio in the protein material of plant tissue. They believed that N and S are assimilated by plants in a proportion similar to the ratio of N to S in the protein, and that in the case of deficiency of one of them, the other nutrient would be accumulated in the plant as non-protein compounds. Therefore, a higher N:S ratio than that occurring in the protein fraction indicates an S deficiency; a lower N:S ratio indicates an S adequacy or, possibly, a deficiency of N.

In a subsequent review of the published data Dijkshoorn and Van Wijk (1967) considered particularly the N:S ratio, and suggested ratios of about 17.5:1 and 13.7:1 as diagnostic N:S values for legumes and grasses, respectively. Subsequent research has shown that the critical N:S ratio for forage legumes should be in the range of 16:1 to 19:1 (Metson, 1973, Spencer et al., 1977b).

The N:S ratio has shown little change with plant age and time and, consequently, has received certain preference for use over the S concentration (Fox et al., 1977; Rasmussen et al., 1977; Robinson and Jones, 1972; Spencer et al., 1977a).

Although based on a known or inferred N:S ratio in the protein fraction, the N:S ratio (total N: total S) is a practical alternative, and is almost invariably used as a diagnostic criterion for S deficiency in forage plants (Metson, 1973).

Forage Responses to Sulfur

Areas with S levels below that needed for adequate plant growth are widespread throughout the world. Crops with S deficiencies have been reported in Europe, Africa, Australia, New Zealand, India, and North, Central and South America (McClung et al., 1959; Coleman, 1966; and Whitehead, 1964). In the United States, by 1981, S deficiencies had been observed in crops grown in 35 States (The Sulphur Institute, 1982).

It has been known that S requirement varies with crops, (Whitehead, 1964; Tandon, 1984). Spencer (1975) classified crops into three categories, i.e., high (group I), moderate (group II), and low (group III) S requirements. Forage species appear in all three groups. Legumes are considered primarily as high or moderate S requirement plants. Alfalfa (group I) and clover (group II) are examples of these two groups. Grasses usually fall in the group II.

Since the study reported here deals with clover, either alone or associated with grass, particular attention was given to the literature on responses to S fertilization by this category of plants.

Clovers Grown Alone

Yield changes in pure stands of white clover stands due to S fertilizations have been reported by several workers. Walker and Bentley (1961) applied 0 or 22 kg S ha^{-1} on alsike clover (Trifolium hybridum L.), red clover (Trifolium pratense L.), and alfalfa (Medicago sativa L.) in 157 locations, representing 18 soil series of Chernozemic and Podzolic soils in West Central Alberta, Canada. They obtained significant yield responses at 72 of those locations where no previous S was added, regardless of soil series and geographical areas.

Subterranean clover (Trifolium subterraneum L.) was grown in some Oregon soils by Dawson (1969) in a greenhouse experiment in which five rates of gypsum (0 to 22 mg kg^{-1}

of soil) were applied. Plant yield and S uptake were significantly increased. The same author used nine locations with established subterranean clover (subclover) stands for field experiments. Sulfur was applied at rates of 0, 11, 22 and 44 kg ha^{-1}, in one initial application or in annual applications for 3 years. Positive yield responses to S fertilization were obtained at all locations. At six of those sites, the annual S applications resulted in significantly higher yields than did one-time fertilization. Rates of S for maximum yields varied considerably with experimental sites (11 kg ha^{-1} in some locations, and up to 44 kg ha^{-1} in others). Highly significant responses to S were reported when soils had less than 7 mg kg^{-1} of phosphate-extractable S, whereas no responses occurred when soils had more than 10 mg kg^{-1}.

Sulfur contents in the plant tissues were also affected by S applications. Jones (1962) applied 0 to 112 mg S kg^{-1} of soil, in a greenhouse study and 0 to 88 kg ha^{-1} in a field experiment with subclover. Significant yield increases, but only slight increases in sulfate concentration in the clover tissues were achieved by application of 11 mg S kg^{-1} soil. However, higher rates than that resulted in an accumulation of S in the plants, essentially in the sulfate form and primarily in the stems.

The effects of S rates (0, 15 and 45 mg kg^{-1} of soil) on clover in two lateritic Podzolic soils and a red-brown earth cropped with subterranean clover in a greenhouse were

studied by Reddy et al. (1981). The three soils had initial $CaCl_2$-extractable S between 8.7 and 10.4 mg kg^{-1} of soil. Sulfur applications did not result in any significant yield changes, but consistently increased S concentration in the plant tissues.

In a greenhouse experiment, Spencer (1959) grew white clover on washed sand under four S rates from 0 to 13 mg kg^{-1} of sand. He found significant increases in yield as well as in N and S concentrations in the plant. The highest S rate gave heavier leaflets, petioles, and stems than did the other rates. The weight of roots was less affected by S treatments than were the plant tops, resulting in wider top: root ratios as the S rate increased.

Spencer et al. (1977b) applied five S rates (0 to 40 mg kg^{-1} of soil) to a sandy loam soil in a greenhouse experiment where subclover was grown. They harvested the legume at three ages, and found significant increases in plant yield due to S applications at all harvests. However, the magnitude of S deficiency increased with progressing time, such that only plants on no-S were deficient at 61 days of growth but all plants except those at the two highest S rates (30 and 40 mg kg^{-1} of soil) were deficient at 133 days. Sulfur concentration in the plant always increased with increasing S rates. At all stages of growth they obtained strong relationships between yield responses to S additions and the N:S ratio.

Tengku Maamun (1985) grew white clover on a Spodosol in a greenhouse study. The treatments included 0 and 60 mg S kg^{-1} of soil. The legume was harvested four times. Forage yield, stolon-root mass, and N and S concentrations in the plant tissue were significantly higher with the S application. The yield decreased progressively with the increase in the number of harvests, and at the time of the fourth harvest most of the plants in the no-S treatment had died. The N:S ratios in the petioles+leaflets and stolon-root tissues of the S-deficient legume were not narrower than 18:1.

The effects of S applications on the N_2-fixing system in Trifolium plants has received particular attention from some workers. Anderson and Spencer (1950) grew subterranean clover on three Australian soils in a series of greenhouse experiments. They found significant beneficial effects of S additions for yield, N and S concentrations, and N and S contents in the legume. They also reported that the increase in clover yield was associated with an increase in the number and size of nodules and the number of larger nodules. They pointed out that limited clover growth was due to the S deficiency which, to a certain extent, restricted symbiotic N_2-fixation.

In a lysimeter study, Shock et al. (1983) grew subclover on a Mollisol which had 8 mg extractable S kg^{-1} of soil. Sulfur was tested at 0 and 37 mg kg^{-1} of soil. Forage yield, S content, and N concentration in the herbage were

significantly increased by S addition. They estimated the amount of N symbiotically fixed in each lysimeter, and concluded that S fertilization increased N_2 fixation by 60% during the first year of the experiment. They also reported that 55% of the S in the legume top came from the fertilizer.

In another lysimeter experiment, Shock et al. (1984a) applied three S rates, 0, 5, and 40 mg kg^{-1} of soil to an Ultisol for subclover. Extractable S in that soil was 7 mg kg^{-1} of soil. This species showed a large yield response to each increment of S. The concentrations of S in the plant tissue showed little change over the no-S treatment with the addition of 5 mg S kg^{-1} of soil, but were doubled with the highest level of added S. At the lower S rates the N:S ratio in the plant top was about 30:1, whereas at the highest S rate it was about 20:1. Recoveries of S from the fertilizer by the plant top were 46 and 17%, respectively, under medium and high rates of S application. In the same experiment, Shock et al. (1984b) reported that S fertilization significantly enhanced the N_2-fixation in subclover. Sulfur applications resulted in significant increase in the amounts of N_2 fixed.

Previous cultivation and fertilization of the soil, soil fallowing, and continuous soil use may influence the legume response to current S applications. Cultivated and uncultivated soils were used by Cowling and Jones (1971) to grow red clover in the greenhouse. Sulfur was supplied at 20

mg kg^{-1} of soil. In the cultivated soil, significant yield increases of the legume were observed. The increases were smaller in the uncultivated soil. Sulfur fertilization increased S concentrations, but decreased N:S ratio in the plant. They related relative yield to the N:S ratio in the plant tissues, and observed that where clover yields were restricted by the S supply the N:S ratio was wider than 25:1. Phosphate-extractable sulfate in the uncultivated and cultivated soils were 17 and 7 mg S kg^{-1} of soil, respectively.

A series of pot experiments with virgin Spodosols (Leon fine sand) was conducted by Harris et al. (1968). Two rates of S, at 0 and 80 mg kg^{-1} of soil, were applied to the soil as gypsum. Sulfur application increased yield, and S and N concentrations in white clover. The effect was very pronounced in the second and third harvests of the legume. The N:S ratio in plants without S treatment was 18:1 at the first harvest, but the values for the second and third harvests were 28:1 and 22:1, respectively. They reported that S-deficient plants produced considerably fewer seeds, indicating the importance of S for the persistance of white clover in permanent swards.

Soils from the southeastern United States were used in a series of 12 greenhouse and 29 field experiments by Jordan and Bardsley (1958). Extractable-S in those soils was 3 mg kg^{-1} of soil or less. Treatments in the greenhouse were with or without S, while five rates of S, 0 to 36 kg ha^{-1}, were

used in the field. Yields of white clover were increased by S additions in all 12 soils in the greenhouse experiments, and the responses were particularly large for the fifth crop. In the field, significant yield responses to S fertilization occurred in 10 experiments. There were no significant differences in yield in the first year of the experiment, but effects became more prevalent as time progressed to the fourth year. They suggested a range from 1.0 to 1.6 g S kg^{-1} in the plant tissue as critical for S deficiency in white clover.

Bardsley and Jordan (1957) grew seven consecutive crops of white clover in seven Mississippi soils with or without the addition of gypsum at a rate of 27 mg S kg^{-1} of soil. In the non-S treatments, yields were significantly lower on three of the soils in the third crop, on all soils in the fifth crop, and the seventh crop was practically a failure. They had stored the soil during summer after the third and the fifth crops. Some S was mineralized during the "fallow" period so the effect of S on yield was not brought about in the fourth and sixth crops. Sulfur and N concentrations in the plant tissue were consistently higher when S was added. The N:S ratio of S deficient plants ranged from 20:1 to 30:1, whereas in the normal plants the range was 10:1 to 17:1.

Field experiments with crimson clover (Trifolium incarnatum L.) in two Florida Ultisols were conducted by Thompson and Neller (1963). One of the soils was virgin,

whereas the other had been cultivated and fertilized with superphosphate for 21 years. Gypsum was applied to supply rates through 36 kg S ha^{-1}. Significant increases in yield and S concentration in clover plants were obtained from the virgin soil but not from the cultivated one. The results seemed to be contrary to those reported by other researchers. This was attributed by those authors to very low extractable S levels (1.2 to 1.4 mg kg^{-1} of soil by Morgan's extractant) in the top 40 cm of the virgin soil, and to the possible existence of adequate amount of residual S in the cultivated and fertilized soil. They also emphasized that for small seeded and shallow-rooted plants, like white clover, S fertilization seems to be necessary for both seedling development and growth to maturity.

Soil moisture supply and its leaching property are directly related to the solubilization of the fertilizer and the depth of the clover root system. Therefore, it affects the response of the legume to S application. These factors need to be taken into consideration in supplying S to establish clover stands.

Williams et al. (1964) conducted a 3-year lysimeter experiment on a sandy loam soil with rose clover (Trifolium hirtum All.). The soil had sodium acetate-extractable S of 20 to 23 mg kg^{-1} of soil and S was applied at 33 mg kg^{-1} of soil either as gypsum or elemental S. The seasonal rainfall was lower in the first and third years than in the second year. As a result of S fertilization, clover yield was

significantly increased in the second year of the experiment, but not in the other two. Where gypsum was applied, S concentrations in the forage were increased for all 3 years, whereas the same occurred only in the last 2 years with elemental S application. At the end of the experiment extractable S ranged between 4 and 12 mg S kg^{-1} of soil in the treatments receiving S fertilizers, and was much lower than in the beginning of the experiment.

Responses of rose clover to S applications at rates of 0, 55, 110, and 165 mg kg^{-1} of soil on lysimeters containing a sandy loam soil were studied by McKell and Williams (1960). Clover yield was significantly higher at the two highest S rates, but the S concentration in the plant tissues did not change significantly. The authors pointed out that 77% of the applied gypsum was leached out of the soil before clover had a chance to initiate its rapid spring growth. Because of high leaching, recoveries by the plants of applied S at rates of 55 and 165 mg kg^{-1} were only 2.8 and 6.7%, respectively.

McLaughlin and Holford (1982) grew white clover in the field on a basaltic soil for 3 consecutive years. In the top 10 cm, the soil had phosphate-extractable S at 4 mg kg^{-1}. Sulfur treatments were of 0, 20, 40, and 80 kg ha^{-1}, and annual fertilization at 10, 20, and 30 kg ha^{-1} for 3 years. Increases in clover yield and S uptake due to S additions were found every year. In the first year maximum yield and S uptake responses occurred at 20 to 30 kg S

ha^{-1}. In the second year, annual S applications resulted in higher plant S uptake, whereas in the third year yield was maximum or near maximum at one single application of 40 kg S ha^{-1} or at three annual applications of 10 kg S ha^{-1}. Gypsum was compared with elemental S, and the former was considered to be superior in terms of plant S uptake when both were applied annually to the sward. The local rainfall during the experimental period was 900 to 1100 mm, and the authors suggested that gypsum had a larger residual effect because the leaching effect on gypsum was smaller.

Andrew (1977) carried out a series of experiments with tropical and temperate pasture legumes, both in the greenhouse and in the field. In the greenhouse, he used three soils with phosphate-extractable S ranging from 0.2 to 2.9 mg kg^{-1}. Sulfur was applied at rates from 0 to 15 mg kg^{-1} of soil. With S applications, both Trifolium repens and Trifolium semipilosum showed significant increases in yield, and in N and S concentrations in the plants. Clovers had maximum yields at S applications of about 5 to 10 mg kg^{-1} of soil. In the field, S rates from 0 to 30 kg ha^{-1} were beneficial to the growth of the Trifolium plants. The author stated that plant response to S application in the field was largely dependent on root morphology and on availability of sulfate in the soil profile. He showed that one had higher extractable S at depths below 30 cm than in the top soil, and pointed out that in such situation white clover (a shallow rooted system) was more responsive to S

additions than a deep-rooted perennial legume. He suggested that 1.8 g S kg^{-1} of plant tissue in the white clover top at the immediate preflowering stage of growth would be a critical level for this element.

Neller (1958) worked with a cultivated Florida soil (Blanton fine sand) on which ordinary superphosphate had supplied a total of 450 kg S ha^{-1} during the 7 previous years. He found that the extractable S (in Morgan's solution) was low in that soil in the top 30 cm. He also indicated that to insure adequate S supply to a shallow rooted legume, the S-containing fertilizer should be applied each fall to prevent the high S leaching in the summer rainfall.

Clover-Grass Mixed Systems

When mixed stands of legumes and grasses are considered it is necessary to keep in mind that one is dealing with plants that have different morphological and physiological characteristics. As a consequence, they have unequal root systems which compete for mineral nutrients and water, as well as unequal plant tops that compete for light. Also, environmental conditions can influence those components of the sward in such a way that either one might be dominant.

In a field experiment where subclover was dominant over the grasses during the spring, Spencer (1978) applied six rates of S (up to 54 kg S ha^{-1}). That fertilization significantly increased the legume yield in the following two

spring seasons, but did not affect grass yield. Clover samples taken from the mixed sward had S concentrations markedly increased by the S application.

Drlica and Jackson (1979) investigated the effects of S applications up to 44 kg ha^{-1}, which were broadcast at each of two fall seasons to a mixture of subterranean clover and annual grasses. Forage yield of the sward was significantly increased by additions of 22 or 44 kg S ha^{-1} in the second year. Sulfur concentration in the legume + grasses significantly increased in both years with added S. A marked increase in the N concentration in the forages occurred in the second year. Clover tissues sampled from the sward had increased S and N concentrations with increasing S rate. A concentration of 2.0 g S kg^{-1} in the clover tops was directly related to the maximum yield of the sward.

A Coastal bermudagrass (Cynodon dactylon [L.] Pers.) - clover (red, crimson, and Ladino) mixture was established in a virgin Florida Ultisol by Thompson and Neller (1963). Extractable S (Morgan's extractant) was 0.9 mg kg^{-1} of soil in the 0 to 15 cm depth, and 29 to 98 mg kg^{-1} of soil in deeper layers down to 90 cm. Annual gypsum applications at rates from 0 to 36 kg S ha^{-1} did not affect significantly the forage yield in the 6 subsequent years. However, significant increases in the forage S concentrations were reported for some harvests in the first and second years of experimentation.

In a virgin Spodosol, Bledsoe and Blaser (1947) applied 0, 66 and 110 kg S ha^{-1} on a mixture of Pensacola bahiagrass, red clover, and medic clover (Medicago lupulina L.). Yields and N and S concentrations in clovers were significantly higher in treatments that received S fertilizer. However, the grass did not show significant changes in the measured parameters.

Adams (1973) overseeded a mixture of clovers on an old grass pasture, where he studied the effectiveness of three S sources, and the frequency of gypsum applications at rates from 0 to 88 kg S ha^{-1}. At least 22 kg S ha^{-1} were needed in the first year for clover establishment in the sward. When more than 22 kg S ha^{-1} was applied in the 4-year period, split applications resulted in higher yield. Over the 4-year period, gypsum, potassium sulfate, and elemental S increased clover yields. Grass yield was increased only in the last 3 years. In the S-fertilized plots, clovers had higher N concentration. This was attributed to the improvement in the N_2-fixation by the legume-bacteria symbiosis. Improvement in N supply was suggested as the main reason for lack of grass response to S additions in the first year and significant response in the subsequent years.

Walker et al.(1956) tested a 30-year old grass pasture (mainly Anthoxanthum odoratum L., Agrostis tenuis Sibth., and Dactylis glomerata L.) for S deficiency, where white clover was sparse and stunted. Five rates of gypsum, through 47 kg ha^{-1} were applied, and the area was oversown with a

mixture of clovers (white, red, subterranean, and alsike clover). Yields, N and S concentrations, and N and S contents in both grasses and legumes were significantly increased by S additions. The yield increase of clovers was much greater than that of grasses. The significant effect of S on grasses was suggested by the authors to be a result of improvement in the N_2-fixation system in the legumes, with subsequent underground transference of N to the grasses.

Neller et al. (1951b) established grass-white clover swards on an Ultisol and on two Spodosols (Rutledge and Immokalee series) where P fertilizer was combined or not with gypsum. Gypsum was supplied at 47 and 60 kg S ha^{-1}, respectively, to the Spodosols and Ultisol. White clover showed significant increases in yield and in N concentration due to S applications. On plots in Spodosols that received no S, growth of white clover was poorer in the second than in the first year of the experiment. Grasses had higher yields in the plots receiving S fertilization, and also showed higher N concentrations where white clover had a good growth in the previous winter.

Working with a Florida Inceptisol, Neller (1952) added phosphates with or without gypsum to a mixture of Pensacola bahiagrass and white clover. Sulfur at 35 kg ha^{-1} was applied in the respective plots each fall for 6 consecutive years. White clover had a good stand where gypsum was added, but was either lacking or very stunted in the no-S treatment. Significant increases in yield and N concentra-

tion were obtained with both the grass and legume. The yield increase of white clover was proportionally larger than that of the grass, and the increase in the N concentration in the grass tissue was considered to be a consequence of the beneficial effects of S fertilization on the legume. The author concluded that "a source of S must be supplied at least once a year for good growth of leguminous forage" in the Florida peninsula.

In a 3-year experiment in a Florida Spodosol, Neller and Bartlett (1959) applied gypsum up to 287 kg ha^{-1} to a mixed sward of Pensacola bahiagrass with Ladino clover (Trifolium repens L.) and sweetclover (Melilotus alba Desr.). In the first year, there were no significant responses to S applications, whereas in the following 2 years significant increases in yield of the grass and legumes were reported. They also described the positive effects of S for the grass as a result of N additions to the system through the legume improvement.

Jones and Martin (1964) applied S every year at rates up to 88 kg ha^{-1} to dryland mixed-pastures in California. They showed significant increase in the yield of subterranean clover, rose clover, and annual grasses. At higher S rates, more S as sulfate accumulated in the forage tissues. Rose clover had low S content when S was applied 2 years before at 22 kg ha^{-1}. In the first year, when N supply was abundant in the soil, there was no difference in sulfate concentrations between clovers and grasses at any S rate.

Two years later, when N supply was inadequate for the growth of the non-legumes, grasses had higher sulfate concentrations than clovers, at every S rate.

Jordan and Bardsley (1958) carried out a series of field experiments with southeastern United States soils. They reported that Coastal bermudagrass had no yield increase in the first year after S applications, but had significant increases in the next 2 years. On the other hand, crimson clover responded positively to S fertilization even in the first year. Subsequently, Jordan et al. (1961) stated that grasses are generally less responsive to S applications than clover, and that grasses are only responsive to such fertilization if they are adequately supplied with N.

Another major point in the S nutrition of the grass-legume mixture is the competition among the forage components for this nutrient (Metson, 1973). Jones (1963) studied the competition among five forage species (subclover + four grasses) growing at five rates of S. Plants were seeded at the same time in pots where S was applied from 0 to 17 mg S kg^{-1} of soil. Subclover and three of the grass species showed significant increase in yield, and sulfate-S concentration as a result of S additions. At no-S treatment, subclover had low yield, and low S concentration, but this legume was very efficient in absorbing and utilizing more S than grasses when this nutrient was made available. Under higher rates of S, growth of the grasses became limited by

low N availability, and they were unable to utilize S. In contrast, subclover having the N_2-fixing system was able to better use the supplied S.

In a field experiment, Jones (1964) studied the effect of simple applications of S at rates of 0, 22, 44, and 88 kg ha^{-1} on a mixed stand of clovers (subterranean, rose, and crimson clover), soft chess (Bromus mollis L.), and harding-grass (Phalaris tuberosa L.). Forage yield was significantly increased by S applications, and the largest increase in yield due to S additions was observed with the clovers. As a consequence, the proportion of clover to grass increased as the rate of S increased up to 88 kg ha^{-1}. The highest S rate also resulted in nearly 15-fold increase in the S uptake by subclover. However, where no S fertilizer was added, the grasses took up much more S than the clovers.

Shock et al. (1983) grew subterranean clover, filaree (Erodium botrys [Cav.] Bertol.), and soft chess (Bromus mollis L.) alone or paired, on a Mollisol in a lysimeter study. Sulfur was applied at 0 and 37 mg kg^{-1} of soil. They reported significant increases in yield, S concentration, and S content in the forages due to S additions. Nitrogen concentration in the plants was higher where S fertilizer was applied to the legume, but not to the grasses. Botanical composition of the mixtures containing subclover shifted toward the legume, when S was supplied. Leaching of S from the fertilizer was smaller in the 2 years of the experiment

where the grass was mixed with subclover than in any grass alone. Subclover took up more S from the fertilizer than any of the grasses.

In another experiment on competition of forage species for S, Shock et al. (1984a) applied 0, 5, and 40 mg S kg^{-1} of soil to an Ultisol where subterranean clover, filaree, and soft chess were grown. Significant increases in forage yield, N and S concentrations, and shift in botanical composition due to S applications were also observed. They reported that in no-S treatments the grass was more competitive for S than subclover; however, at the highest S rate the legume had a stronger competition position. The authors pointed out that S was a limiting nutrient to subclover under no-S applications, but N was the great limitation to the grass mixed with clover at the highest S rate, where the legume was able to get enough N through the N_2-fixation system.

Sulfur fertilization to clover alone or mixed with grasses has resulted in significant increases in yield, and S concentration and content in the legume. Beneficial effect to the N_2-fixation and/or N metabolism in the legume has been observed. Grasses are said to compete for S with the legume. Not many studies have presented data on the N:S ratio for the legume and the grass, and on the extractable S in the soil. Very few workers have reported effects of S applications on white clover-Pensacola bahiagrass mixture.

Turning the focus to the S applications to such mixture maintained over several years, the information is completely lacking. Studies on the S x Mo interaction on white clover represent another gap in the literature.

Nutrient Distribution in the Soil Profile

The movement of nutrients in the soil profile is a result of a combination of several factors such as climatic conditions, type of vegetative cover, specific mobility of the nutrients, characteristics of the soil, and amendment and fertilization program adopted in the area.

Generally, the higher the annual rainfall and the more open plant canopy, the higher the downward movement of nutrients in the soil profile.

Tisdale et al. (1985) described Ca, Mg, K, and S as mobile nutrients that can be easily leached out of the soil profile. On the other hand, P is the least mobile macronutrient and usually tends to accumulate close to the soil surface (Ozanne, 1980; Tisdale et al., 1985). Stevenson and Ardakani (1972) suggested that Cu, Fe, Mn, and Zn present little downward movement in the soil.

Physical and chemical characteristics of the soil are well-known to affect nutrient movement in the profile. Soil texture, drainage, and permeability influence water movement throughout the profile and the distribution of nutrients in the soil. Severe leaching of nutrients may occur in sandy

soils located at places under heavy rainfall conditions (Tisdale et al., 1985).

Coleman et al. (1958) have written Ca and Mg movement in the soil depends on the exchange characteristics and the anion accompanying both nutrients. Longnecker and Sprague (1940) limed a soil with apparently low permanent negative charge and three soils with substantial amount of permanent negative charges. They found that Ca accumulated in the top 2.5 cm in the low charge soil, whereas the nutrient moved downward in the other three soils. Pavan et al. (1984) applied $CaCO_3$ in the top 2 cm of an Oxisol placed in columns, and found that Ca accumulated in the top 10 cm of the soil profile. They attributed the low movement of Ca in the profile to the lack of a stable anion to promote the downward movement of soluble cation and to a possible low solubility of $CaCO_3$.

Adsorption of nutrients occurring as anions, such as P and S, on surface of soil particle can play an important part in determining their distribution in soil profiles. The amount of anion that is adsorbed depends on the clay mineralogy, clay content, soil pH, anion concentration, presence of other anions in the soil (Sanchez and Uehara, 1980; Tisdale et al., 1985). Sanchez and Uehara (1980) pointed out that acid Oxisols, Ultisols, and Andosols are able to adsorb large amounts of P.

Rates and combinations of amendments have been shown to affect the distribution of extractable Ca and Mg, and soil

pH in the profile. Lutz and Jones (1974) found that dolomitic lime at 0, 22.4, and 44.8 Mg ha^{-1} applied to the top (0 to 30 cm) of an Ultisol increased soil pH in the 0 to 30 cm and 30 to 45 cm soil depth. Below those limits, no substantial effect of lime on soil pH was observed. Available Ca in the soil increased with increasing lime rates. Available Mg appeared to show greater downward movement at the highest lime rate. Fernandez (1983) also found significant increases in subsoil pH, and extractable Ca and Mg as a result of application of lime and lime-gypsum to the surface of an Entisol and an Ultisol.

Reeve and Sumner (1972) reported substantial amounts of Ca in the subsoil (down to 45 cm) of an Oxisol that had received surface-applied lime. When they used gypsum in combination with lime they found further downward movement of Ca in the profile. Pavan et al. (1984) found significantly higher extractable Ca throughout the 0 to 100 cm depth of an Oxisol placed in columns, when gypsum was applied than with $CaCO_3$ application. Both experiments demonstrated that extractable Mg was decreased throughout the soil profile when grupsum was used.

Micronutrients movement and distribution in soil profiles are not extensively studied. For being bound to organic substances in the soil, Cu, Fe, Mn, and Zn may have their mobility subject to the movement of the formed complexes (Stevenson and Ardakani, 1972).

Smith et al. (1962) found that higher pH in the topsoil or in the subsoil of the Lakeland fine sand (Entisol) resulted in higher leaching of Cu, Mn, and Zn. They observed that Cu was the least and Mn was the most mobile of those three micronutrients. Also, Jones and Belling (1967) reported that Cu remained in the 3 cm of surface soil when seven soils were placed in columns, received Cu fertilizer, and were leached.

The placement of fertilizers in soils can affect nutrient distribution in the profile. It is variable with the crop grown or land use. For permanent pasture establishment, liming material is usually broadcast and incorporated into the top 15 to 30 cm of the soil whereas fertilizers are mainly added to furrows or only broadcast with forage seeds. Subsequently, both lime and fertilizer are periodically applied to the soil surface.

Adams et al. (1967) applied 0, 7.8 and 40.3 Mg dolomitic limestone ha^{-1} to an acid Ultisol where coastal bermudagrass (Cynodon dactylon [L.] Pers.) was planted. Lime was surface-applied or mixed with 0 to 15 cm soil. After 3.5 years they sampled the soil by 15-cm increments from the surface down to 120 cm depth. They observed that the surface liming at 7.8 or 40.3 Mg ha^{-1} had not increased soil pH appreciable below the 15 m depth. Limestone mixed with the soil increased soil pH to the 30 or 60 cm depth, depending on the N rate applied. Extractable Ca + Mg were

markedly increased to the 30-cm soil depth by the highest lime rate.

Blue (1970) reported that 70% of the P supplied (mostly surface-applied) over an 18-year period to established white clover-Pensacola bahiagrass pastures on Spodosols had remained in the 15 cm of surface soil. Ozanne (1980) stated the the bulk of the P applied to the surface soil on swards and pastures is retained in the surface 2 to 3 cm of soil.

Koger et al. (1970) sampled seven layers from the surface down to 53 cm of a Spodosol where clover-grass pastures had been limed and fertilized for 9 years. They found substantial accumulation of ammonium acetate-extractable Ca and K as well as much higher soil pH in the top 15 cm of the soil. Phosphorus was mainly retained in the surface 7.5 cm of soil.

CHAPTER III
RESPONSES TO SULFUR FERTILIZATION BY A WHITE CLOVER-PENSACOLA BAHIAGRASS SWARD

Introduction

White clover (Trifolium repens L.) - Pensacola bahiagrass (Paspalum notatum Flugge) pastures have been shown to be economically feasible for beef cattle operations on Spodosols in North Central Florida (Koger et al., 1961; 1977).

A white clover-Pensacola bahiagrass sward had been productive for 25 years under periodic liming, sporadic B, Cu, Fe, Mn, and Zn supply, and annual P and K applications (Blue, 1979). However, white clover has not performed well in recent years, even under such management. Ordinary superphosphate was applied in the first 13 years, but afterward concentrated superphosphate was used to supply P and no S was included in the fertilizer.

Mitchell and Blue (1981) showed that phosphate-extractable S was very low (ranging from 1 to 8 mg S kg^{-1} of soil) throughout the profile of nine Florida Spodosols of which two were Myakka fine sand.

Forage responses to S applications commonly occur when the extractable S in the soil is below 10 to 12 mg kg^{-1} of

soil. Grasses, which have a lower S requirement than legumes, also are very competitive with legumes for S (Fox et al., 1964; Jones, 1963; Jordan et al., 1961; Reisenauer et al., 1973).

Under moderate or heavy rainfall, S from gypsum or potassium sulfate applied to sandy soils has leached from the soil profile in appreciable amounts (Barrow, 1966; Chao et al., 1962; Watson, 1969). As a consequence, the recovery of S by plants from the fertilizer has been very low in such situations (McKell and Williams, 1960; Shock et al., 1983).

Mitchell (1980) reported that when S was applied to bahiagrass in the spring, stolon-root tissues sampled the following fall had higher S concentrations. Also, preliminary data for the clover-grass sward used by Blue (1979), showed that bahiagrass had higher S concentrations than the accompanying white clover. Whether the higher S concentration in the top of the grass was a result of S storage in the stolon-root tissues during a period of slow plant growth was still a remaining question.

The principal objectives of this study were: a) to determine the effect of S application on forage yield, S concentration in the plant tissue, S uptake by the plants, and S recovery from the fertilizer during a 4-year period in the sward where white clover had been present for 28 years (experiment no. 1), and in the sward where white clover was oversown at initiation of the present study (experiment no. 3), and b) to determine whether winter-applied S is

stored in the Pensacola bahiagrass stolon-root tissues during the spring (experiment no. 2).

Materials and Methods

Pensacola bahiagrass and white clover were seeded in 1952 on virgin Myakka fine sand (sandy, siliceous, hyperthermic Aeric Haplaquod) at the Beef Research Unit near Gainesville, Florida.

Two sets of experimental plots located in that field were used in the present studies.

Experiment No. 1

Forty plots of 41 m^2 each were established in 1952 to study the effects of lime and mixtures of micronutrients on the mixed sward. There were 10 treatments and four replications. One treatment was unlimed, and the other nine received lime at 5 Mg ha^{-1}. One of the treatments that included lime did not receive any micronutrient-containing fertilizer. The remaining eight treatments were fertilized with a mixture of Cu, Fe, Mn, Mo, and Zn combined with a variety of B sources. Micronutrient combinations were applied from 1952 through 1959. A mixture of B, Cu, Fe, Mn, Mo and Zn was inadvertently applied to all plots in 1973. After the initial lime application, additional lime had been applied to maintain the surface soil (0 to 15 cm) near pH 6.0. Phosphorus and K were broadcast annually on all plots. Forages were harvested five times a year and removed

from the area (Blue, 1979). Only limed plots (nine treatments originally identified as no. 2 through 10), and, therefore, having white clover associated with the grass, were used for the present experiment. Hereafter, those 36 plots will be designated as Sward I.

In November 1980, each plot was split into two subplots having an area of 20.5 m^2. One subplot received no S fertilization; gypsum was broadcast at a rate of 40 kg S ha^{-1} on the other. At the same time, 20 kg P ha^{-1} as concentrated superphosphate, and 70 kg K ha^{-1} as KCl were applied uniformly to all plots. Sulfur and K were reapplied as K_2SO_4 and KCl in April 1981, at rates of 30 and 90 kg ha^{-1}, respectively. In November 1981, another 40 kg S ha^{-1} as gypsum were applied together with 40 kg P ha^{-1} as concentrated superphosphate and 140 kg K ha^{-1} as KCl. The same amounts and sources of P and K as in 1981 were applied in November 1982, when the white clover 'Osceola' was oversown. At the same time, 1.1 Mg dolomitic limestone ha^{-1} were also applied. Phosphorus at 51 kg ha^{-1} as concentrated superphosphate, and K at 240 kg ha^{-1} as KCl or K_2SO_4 were split into three equal portions and applied in November 1983, and January and March 1984. Sulfur was supplied to S subplots as K_2SO_4 at a total rate of 90 kg ha^{-1}.

Forages were harvested four times per year in May, late June, August, and October. Plant samples were collected from each subplot, and dried at 70° C.

At the time of the first harvest (May) in 1983, and first (May) and second (late June) harvests in 1984, pure white clover samples were picked from 32 subplots (16 with S and 16 without S fertilization). The number of petioles was counted in each sample. Samples were dried at 70° C, and weighed.

Plant samples were ground to pass a 0.85-mm stainless-steel screen in a Wiley mill. Total N was determined by a semimicro-Kjeldahl procedure, with salicylic acid and sodium thiosulfate to include nitrate and nitrite in the determination. In preparing extracts for Ca, Mg, K, P, Cu, Fe, Mn, and Zn analyses, 1 g of plant tissue was dry ashed, and the ash was digested with 20 mL of 6 M HCl. The solution was evaporated to dryness, and the residue was dissolved in 2.25 mL of 6 M HCl. The new solution was heated, 10 to 15 mL deionized water was added, and the suspension was filtered. The filter was washed several times with deionized water, and the filtrate was made to 50 mL volume. Calcium, Mg, Cu, Fe, Mn, and Zn were determined by atomic absorption spectrometry, and K by emission spectrometry. The ammonium molybdate/ascorbic acid colorimetric method was used to analyse P, which was determined in the Technicon Auto Analyzer II. Total S determination was done by direct combustion of the plant tissue under O_2 in a Leco-132 Sulfur Determinator with an infrared detector (Leco Corporation, 1980).

Soil samples were collected at a depth of 0-15 cm in May 1984. Samples were air dried, and sieved through a 2-mm

screen. Soil pH was determined in 1:2 (w/v) water and 1 M KCl suspensions. For Ca, Mg, K, and P determinations, soil samples were extracted with Mehlich-I double-acid extractant (Nelson et al., 1953). Sulfur was extracted from 10 g of soil with 25 mL of a 500 mg P L^{-1} as Ca $(H_2PO_4)_2.2H_2O$ solution, and determined turbidimetrically (Butters and Chenery, 1959; Massuomi and Cornfield, 1963). Soil samples were ground to pass a 0.5-mm screen before carrying out organic C, and total N and S determinations. Organic C was determined by the Walkley-Black procedure as modified by Walkley (1947); N was determined by the procedure described by Nelson and Sommers (1972), and total S was obtained via infrared analysis after direct combustion of the sample in the Leco SC-132 Sulfur Analyzer.

The rainfall data recorded at the Beef Research Unit for the November 1980 to October 1984 are presented in Fig. 3-1.

Analysis of variance and mean comparisons by Duncan Multiple Range Test were performed according to procedures given by Freund and Littell (1981).

Experiment No. 2

In May 1984, cylindrical cores (12.5-cm diameter and 15.0-cm deep) were taken to sample stolons and roots from the 32 subplots (16 fertilized with S, and 16 without S fertilization) corresponding to the four replications of the

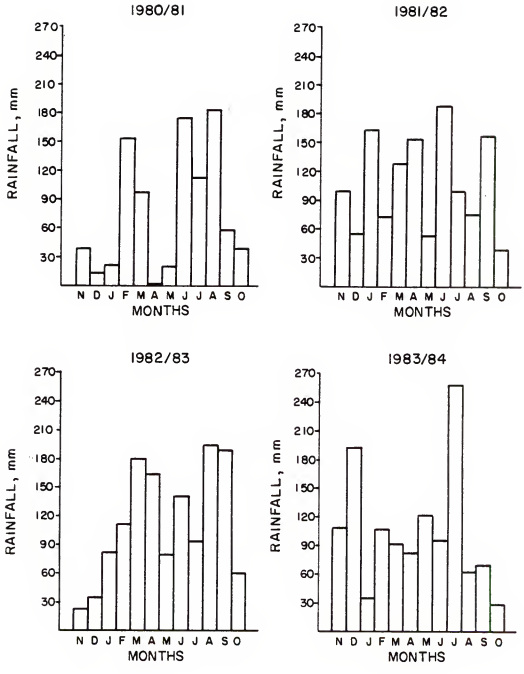

Fig. 3-1. Monthly rainfall recorded at Beef Research Unit from November 1980 to October 1984.

treatments originally identified as No. 4, 6, 7 and 8 in Sward I. Stolon-root material was immediately separated from the soil, thoroughly washed with tap water, and then washed with deionized water. Plant material was dried at 70°C, weighed, and ground to pass a 0.85-mm stainless screen in a Wiley mill.

Nitrogen, S, Ca, Mg, K, and P were determined in the stolon-root material following the same procedures described for plant tissue in Experiment No. 1. Statistical analysis followed SAS statements presented by Freund and Littell (1981).

Experiment No. 3

Another 96 experimental plots having 14 m^2 each were established in 1963 near the plots designated as Sward I. They represented four replications of 24 (4x3x2) treatments resulting from the combinations among rototilling (none, annually, biennially, triennially), lime sources (calcic or dolomitic lime at 2.5 Mg ha^{-1}), and N rates (0, 55, 110 kg N ha^{-1} as NH_4NO_3). Phosphorus and K were broadcasted annually at 40 and 150 kg ha^{-1}, respectively. In 1977, dolomitic lime was broadcast on all plots, at 2.2 Mg ha^{-1}. Forages were periodically mowed and removed from the area. In November 1982, white clover 'Osceola' was oversown in all plots, which were very homogenous in terms of forage growth. This set of plots was used for this study and will hereafter be referred to as Sward II.

Three rates of S (0, 45, and 90 kg S ha^{-1}) as K_2SO_4 were applied. Those rates were split into three equal quantities applied in November 1983, January, and March, 1984. For each application, K_2SO_4 was mixed with 17 kg P ha^{-1} as concentrated superphosphate. Potassium chloride was added to the mixture applied to plots receiving 0 or 45 kg S ha^{-1} in order to supply 80 kg K ha^{-1} each time. As a total of the three applications, P and K were supplied at 51 and 240 kg ha^{-1}, respectively.

Treatments were assigned in randomized complete block design, and each S rate was replicated 32 times.

Forage was harvested four times during 1984 in May, late June, August, and October. Plant samples were collected from each plot at each harvest.

Twenty-four plots (8 for each S rate) were chosen for sampling of pure white clover. Those samples were obtained at the first and second harvests. The number of petioles was counted, and after drying at 70°C, the samples were weighed.

Plant samples were analyzed for total N, total S, Ca, Mg, K, P, Cu, Fe, Mn, and Zn following the procedures described for Experiment No. 1. Data were statistically analyzed according to Freund and Littell (1981).

Results and Discussion

Experiment No. 1

Sulfur applications to the Spodosol resulted in significant increases in forage yield, and N and S contents

in the mixed sward every year during the 1981-1984 period (Table 3-1). These increases were a consequence of the significant and positive effects of S fertilization in 14 out of 16 harvests. The magnitude of yield, and N and S contents increases was greater in 1981 and 1984 than in other years, and followed applications of larger amounts of S fertilizers. Beneficial effects of S applications for clover and grasses were reported by Harris et al. (1968), Jones (1964), Neller (1952), Shock et al. (1983), and Woodhouse (1969).

Table 3-1. Annual forage yield, and N and S contents in white clover + Pensacola bahiagrass in Sward I.

Year	Sulfur	Forage		
		Yield	N	S
		----------kg ha^{-1}----------		
1981	No	9840b*	173b	19.0b
	Yes#	11530a	215a	33.4a
1982	No	8770b	113b	17.9b
	Yes	9590a	124a	21.9a
1983	No	11100b	160b	20.2b
	Yes	12070a	174a	23.1a
1984	No	9030b	118b	16.4b
	Yes	11230a	151a	23.1a

#Sulfur applied in 1980, in 1981, and in 1983/84.
*Means within columns in each year followed by the same letters are not significantly different at the 5% level of probability (DMRT).

As a cool-season legume, white clover is expected to be the dominant species in the sward during the winter, and to

some extent during the spring. Based on N concentration data (Table 3-2), white clover was indeed a major component of the sward only in the winter-spring of 1981. Nitrogen concentration in the plant tissue at that time was increased as a result of the S fertilization. The higher N concentration was responsible for an increase of greater magnitude in N content of the forages than in the forage yield in 1981 (Table 3-1). Beneficial effects of S fertilization on N_2-fixation and/or N metabolism in forages were reported by Anderson and Spencer (1950), Cairns and Carson (1961), Robson (1978) and Shock et al. (1983). Also, in the forage harvested in May, 1983, the N concentration was relatively higher than at the other harvests. White clover plants were relatively abundant in the sward at that time. In most of the August and October harvests, when Pensacola bahiagrass was dominant in the stand, the N concentrations in the forage ranged between 10.4 and 13.7 g kg^{-1}. These concentrations are close to the 13.4 g kg^{-1} reported by Blue (1979) for Pensacola bahiagrass grown in unlimed plots where white clover normally did not develop.

Sulfur concentrations in the forages from plots that received S fertilizer were significantly higher in all harvests in 1981, in two harvests in 1983, and in three out of the four harvests in 1984 than in forages without S. The increase in S concentration followed the application of the higher S rates (70 and 90 kg S ha^{-1}) used in the experiment in 1981 and 1984. Also, the rainfall following the S

fertilization in 1981 was not high (73 mm) in the November through January period, so that forages (especially white clover) could grow and take up S before leaching. Although the amount of rain was much higher (336 mm in an equivalent period) late in 1983 and early 1984, S leaching was minimized because it was supplied in three applications.

In the first forage harvest both in 1981 and 1983, when white clover made appreciable contributions to the mixed sward, the S concentrations in the forages were the lowest in the experiment. Since the N concentrations in the forages at both harvests were the largest, the widest N:S ratios were recorded in the mixed sward (Table 3-2). However, at those harvests when bahiagrass was the main component in the sward, forages had the highest S concentrations, the lowest N concentrations, and N:S ratios ranging between 3.5:1 and 8.9:1. Similar low N:S ratios in Pensacola bahiagrass were reported by Mitchell (1980). Dijkshoorn and Van Wijk (1967) suggested a N:S ratio of 13.7:1, whereas Metson (1973) presented 1 to 1.5 g S kg^{-1} plant tissue as adequate values for forage grasses. Pensacola bahiagrass apparently absorbed S to satisfy plant needs, and even to accumulate S in the plant tissues. In such situations, probably the amount of N absorbed by the grass limited protein synthesis, and did not allow plants to keep that suggested 13.7:1 ratio nearly constant. Nitrogen insufficiency for grass receiving S fertilization has been reported by Anderson and Spencer

Table 3-2. Forage N and S concentrations, and N:S ratio in white clover-Pensacola bahiagrass in the Sward I, from 1981 to 1984.

Harvest time	Sulfur	1981			1982			1983			1984		
		N	S	N:S	N	S	N:S	N	S	N:S	N	S	N:S
		---g kg^{-1}---		ratio	---g kg^{-1}---		ratio	---g kg^{-1}---		ratio	---g kg^{-1}---		ratio
May	No	25.1b*	1.45b	16.9a	17.0a	2.31a	7.4a	19.3a	1.45a	14.2a	16.0a	1.73b	8.9a
	Yes#	28.5a	1.86a	15.4b	17.3a	2.38a	7.3a	19.0a	1.47a	13.5a	15.8a	1.84a	8.8a
Jun	No	16.4a	2.13b	7.7a	10.8a	1.91a	5.6a	14.9b	1.87b	8.1a	14.4b	1.82b	7.9a
	Yes	16.4a	3.42a	4.7b	10.6a	2.03a	5.2b	15.4a	1.99a	7.9a	15.4a	2.03a	7.4b
Aug	No	13.7a	2.65b	5.4a	12.6a	2.10a	6.1a	11.6a	1.81b	6.5a	10.4a	1.85a	5.6a
	Yes	12.9b	3.76a	3.5b	12.7a	2.18a	6.0a	11.7a	1.93a	6.2b	10.7a	1.93a	5.6a
Oct	No	13.2a	2.07b	6.6a	12.6a	2.31a	5.6a	11.5a	1.98a	6.0a	12.1b	1.92b	6.4a
	Yes	13.2a	2.57a	5.2b	12.4a	2.35a	5.3a	11.7a	2.01a	5.9a	12.4a	2.04a	6.1a

#Sulfur applied in November 1980, April and November 1981, November 1983, and January and March 1984.

*Means within columns at each harvest followed by the same letters are not significantly different at the 5% level of probability (DMRT).

(1950), Jones and Martin (1964), Jordan et al. (1961), and Terman et al. (1973).

Pensacola bahiagrass accumulated S, and the increase in S content in the sward was of greater magnitude than the increase in forage yield from fertilization (Table 3-1). Plants growing in plots fertilized with S had S contents that exceeded those in the control plots by 26.5 kg S ha^{-1} in the 4 years of the experiment. This value corresponded to an apparent S recovery of 13.2% over that period. Presumably, leaching would be mainly responsible for the S loss. Neller (1952) pointed out that S can be readily leached out of the root zone of established forages in sandy soils of Peninsular Florida, and leaching has been reported as one of the major processes for S losses (Barrow, 1966; McKell and Williams, 1960; Shock et al., 1983).

When the first sampling of white clover was made in 1983, no significant effect was found from previous S fertilizations on macronutrient or micronutrient concentrations (Table 3-3). Sulfur concentrations in the legume were below the suggested critical range of 1.5 to 2.1 g kg^{-1} (Metson, 1973). The N:S ratios were wider than the 17.5:1 suggested by Dijkshoorn and Van Wijk (1967) as adequate for legumes. White clover did not absorb S in an amount sufficient to meet plant requirements, and consequently was S-deficient.

In 1984, the white clover population in the mixed sward was small. Data in Table 3-2 illustrate the meager popula-

tion through the N concentration in the plant samples from the mixed sward.

Table 3-3. Macronutrient and micronutrient concentrations and N:S ratio in the white clover sampled in the Sward I in May 1983.

Response variables	S fertilization	
	No	Yes#
N (g kg^{-1})	29.9a*	29.8a
S (g kg^{-1})	1.32a	1.34a
N:S (ratio)	22.7a	22.4a
Ca (g kg^{-1})	12.6a	12.1a
Mg (g kg^{-1})	3.1a	2.8a
K (g kg^{-1})	29.0a	27.5a
P (g kg^{-1})	3.9a	3.9a
Cu (mg kg^{-1})	5.9a	5.8a
Mn (mg kg^{-1})	34a	35a
Fe (mg kg^{-1})	62a	62a
Zn (mg kg^{-1})	29a	28a

#Sulfur applied in 1980, and 1981.
*Means within rows followed by the same letters are not significantly different at the 5% level of probability (DMRT).

The occurrence of severe frosts in the area toward the end of December 1983 and January 1984, severely damaged forages in the sward. White clover plants in the sward did not develop normally, and were stunted (usually less than 5 cm tall) at both first and second harvests. The average individual weight of petioles + leaflets was significantly higher in plants growing in plots that received S fertilization, and the magnitude of this S effect was particularly noticeable in the May 1984 sampling (Table 3-4). Sulfur concentration, and N:S ratio in the legume sampled in May or June indicated that the surviving plants in the mixed sward

had an adequate S supply. Although the magnitude of changes was not so great, and probably was not of major consequence, S application resulted in significant increases of N, S, Ca, Mg, and Cu concentrations in the plant tissue. However, significant decreases in K concentration of white clover were observed at both sampling times (Table 3-4). Decreases in K concentration in forages as a result of S fertilizations have been reported by Bledsoe and Blaser (1947), Harris et al. (1968), and Ulrich and Hylton (1968).

Table 3-4. Average (petiole + leaflets) weight, macronutrient and micronutrient concentrations, and N:S ratio in white clover sampled in the Sward I in May and in June 1984.

Response variables	S fertilization			
	No	Yes#	No	Yes
	May 1984		June 1984	
Weight (mg)@	45.6b*	61.5a	21.0b*	22.7a
N (g kg^{-1})	30.4a	29.8a	29.4b	31.2a
S (g kg^{-1})	1.67a	1.63a	1.94b	2.02a
N:S (ratio)	18.2a	18.2a	15.1a	15.5a
Ca (g kg^{-1})	15.2b	19.6a	18.1a	18.6a
Mg (g kg^{-1})	4.7b	5.8a	4.8b	5.5a
K (g kg^{-1})	37.2a	19.1b	30.5a	21.4b
P (g kg^{-1})	3.1a	3.3a	3.2a	3.4a
Cu (mg kg^{-1})	4.6a	4.9a	3.9b	4.2a
Fe (mg kg^{-1})	69a	65b	75a	72a
Mn (mg kg^{-1})	36a	34a	33a	31a
Zn (mg kg^{-1})	23a	21b	24a	24a

#Sulfur applied in 1980, in 1981, and in 1983/84.
@Average weight of each petiole with three leaflets.
*Means within subtables rows followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Phosphorus and the micronutrients, Cu, Fe, Mn and Zn, were monitored in white clover samples. According to the

critical levels suggested by Martin and Matocha (1973) and McNaught (1970) none of these nutrients seemed to be deficient for the legume. As pointed out by those authors, and by Tengku Maamun (1985), only Cu concentration was marginal, and would deserve attention in future fertilization.

Chemical properties of the top soil collected in May 1984 are presented in Table 3-5. Soil pH determined either in water or in 1 M KCl, and extractable Ca, Mg and P were not significantly changed by the S applications. However, in a similar way to K concentrations in white clover sampled in the sward in 1984 (Table 3-4), extractable K in the top soil was significantly lower in the plots where S had been applied than in the no-S treatment. Tengku Maamun (1985) showed a similar result with soil extractable K. Phosphate-extractable S had statistically identical values in the top soil of plots receiving no-S and in those fertilized with a total of 200 kg S ha^{-1} over the 4 years (Table 3-5). This confirmed that S was either taken up by forages or was lost from the top layer of the soil. That net S immobilization over the entire period had not occurred in the top soil is confirmed by the fact that total S was not increased in the S-fertilized plots. Net mineralization of organic matter occurred in the top 15 cm layer of the S-treated soil as evidenced by significant decreases in the organic C and total N in soil from that treatment. The magnitude of C loss was higher than the N mineralized, so that the C:N and C:S ratios were significantly more narrow in the top soil of the

S-fertilized plots than in those without S. The N:S ratio in the soil was not changed by the S treatments. Stewart (1969) mentioned that as organic matter is decomposed, N and S are mineralized in the same ratio as the total N: total S in the soil. In fact, in the present study, the soil had a total N: total S ratio of 7.5, and one part of S was mineralized for every 8 parts of N mineralized.

Table 3-5. Some soil chemical properties determined in samples collected (0-15 cm) in the fourth year of the experiment with Sward I.

Soil properties	S fertilization	
	No	Yes#
pH (water, 2:1)	5.9a*	5.8a
pH (1 M KCl, 2:1)	5.2a	5.2a
Organic C (g kg^{-1})	28.1a	22.8b
Total N (mg kg^{-1})	1356a	1240b
Total S (mg kg^{-1})	180a	166a
Organic C: total N	20.8a	18.5b
Organic C: total S	156a	138b
Total N: total S	7.5a	7.5a
Extractable Ca (cmol $[p^+]$ kg^{-1})	7.72a	6.71a
Extractable Mg (cmol $[p^+]$ kg^{-1})	1.41a	1.31a
Extractable K (cmol $[p^+]$ kg^{-1})	0.18a	0.07b
Extractable P (mg kg^{-1})	66a	59a
Extractable S (mg kg^{-1})	4.6a	4.1a

#Sulfur applied in 1980, in 1981, and in 1983/84.
*Means within rows followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Experiment No. 2

The mass of the stolon-root system of Pensacola bahiagrass did not show any significant change due to S fertilization (Table 3-6). Neither the concentrations of N,

S, P, Ca, and Mg, nor the S contents in the stolon-root tissue was statistically different between S-treated and non-S-treated subplots.

Table 3-6. Mass, macronutrient concentrations, N:S ratio, and N and S contents in the stolon-root tissue of Pensacola bahiagrass collected@ in the Sward I in May 1984.

Response variables	S fertilization	
	No	Yes#
Mass (kg ha^{-1})	15160a*	15570a
N (g kg^{-1})	8.2a	8.7a
S (g kg^{-1})	2.03a	2.11a
N:S (ratio)	4.1a	4.1a
N content (kg ha^{-1})	123b	134a
S content (kg ha^{-1})	31a	33a
Ca (g kg^{-1})	5.4a	5.5a
Mg (g kg^{-1})	1.7a	1.8a
K (g kg^{-1})	5.3a	3.9b

@A cylindrical core with 12.5 cm diameter and 15 cm height was used. One hectare is equivalent to 815,000 cores.
#Sulfur applied in 1980, in 1981, and in 1983/84.
*Means within rows followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Mitchell (1980) reported that applications of 40 kg S ha^{-1} to Pensacola bahiagrass growing on Myakka fine sand resulted in significant increases in S concentrations and contents in the stolon-roots of the grass. The figures he reported for S concentrations (1.25 to 1.85 g kg^{-1}) were much lower than those presented in Table 3-6. Mitchell's stolon-root samples were taken in the fall season, whereas samples of this study were collected in May. Furthermore, Blue and Graetz (1977) and Impithuksa and Blue (1977) have shown that N concentration and content in stolon-root tissue

of bahiagrass commonly vary with season. This variation might also be applicable to S.

Sulfur accumulation in the stolon-root system of Pensacola bahiagrass could not be proven by these data. On the other hand, this grass has deep roots (Ward and Watson, 1973), and as will be discussed later (Fig. 6-3), concentration of the phosphate-extractable S tended to increase with soil depth below the top 15 cm and was markedly higher at the 75 to 90 cm depth than elsewhere in the profile. Bahiagrass may have absorbed appreciable amounts of S through its deeper roots.

The N:S ratio in the stolon-root tissue was equal to 4.1:1 (Table 3-6) in both control and S-treated plots. This was about one half of the ratio in the plant tops (Table 3-2) for the May 1984 harvest when bahiagrass was dominant in the sward. Mitchell (1980) presented ratios of 4.5:1 to 5.6:1 in the stolon-root tissue of this grass that received 200 kg N ha^{-1}, with or without S fertilization.

Nitrogen content in the stolon-root tissues was significantly higher for plants receiving S than for those without S. As presented in Table 3-5 (Experiment No. 1), net N mineralization in the top soil was more evident in the S-treated plots than in those without S. Bahiagrass stolon-root tissues should have absorbed nore N where N mineralization was higher. Through relatively small (and non-significant) increases in both mass and N concentrations, significant increase in N content in this tissue was observed.

Stolon-root tissues of bahiagrass grown in plots that received S fertilizer had significantly lower K concentration than those without S.

Experiment No. 3

The significant increase in 1984 total forage yield was mainly due to the effect of 90 kg S ha^{-1} application on the forage yield obtained in the first harvest (Table 3-7). White clover had few plants in the sward all year because of an earlier freeze, and did not make much contribution to the sward even at the time of May or June harvests.

Nitrogen content in the forages was also increased due to 90 kg S ha^{-1} applications, when all harvests or only those of May and June are considered. Concentrations of N in forages harvested in May and June were significantly increased by S application at a rate of 45 kg ha^{-1}. These effects may have been a result of greater N_2-fixation due to S fertilization of the thinly populated legume and/or the N assimilation of both grass and legume (Anderson and Spencer, 1950; Cairns and Carson, 1961; Robson, 1978; Shock et al., 1983).

Sulfur application at 45 kg ha^{-1} resulted in significantly higher S concentration in the forages throughout the year. Sulfur contents in the forages, especially on an annual basis, was significantly increased over the control treatment by S fertilizations. Forages harvested in the plots at S rates of 45 and 90 kg ha^{-1} contained 3.3 and 4.4

kg ha^{-1}, respectively, more S than that of no S treatment. These figures represent apparent S recoveries of 7.3 and 4.8%, respectively, which are very low. As previously discussed (Experiment No. 1), large loss of S had taken place in this soil probably by leaching (Barrow, 1966; McKell and Williams, 1960; Neller, 1952; Shock et al., 1983). Shock et al. (1984a) reported a large reduction in the percentage of S recovery by forage tops as the S rate increased through 80 kg ha^{-1}.

Table 3-7. Forage yield, N and S concentrations, N:S ratio, and N and S contents in white clover + Pensacola bahiagrass in the Sward II, for each harvest and on an annual basis.

Harvest time	S rates	Forage					
		Yield	N	S	N:S	N	S
	kg ha^{-1}	kg ha^{-1}	----g kg^{-1}----		ratio	--kg ha^{-1}--	
	0	2320b*	14.6b	1.98b	7.4a	34b	4.5b
MAY	45#	2410b	15.4a	2.69a	5.6b	37b	6.1ab
	90	3410a	13.8c	2.00b	6.7a	47a	7.2a
	0	1420a	14.0b	1.87b	7.3a	20b	2.6a
JUNE	45	1470a	14.5ab	2.25a	6.4b	21ab	3.2a
	90	1560a	14.7a	1.94b	7.4a	23a	3.0a
	0	3060a	11.2a	2.05b	5.5a	34a	5.7b
AUG	45	3140a	11.1a	2.28a	4.8b	35a	6.5a
	90	3240a	11.3a	2.11b	5.3a	37a	6.8a
	0	2000a	12.4a	1.86b	6.7a	25a	3.6a
OCT	45	1990a	12.4a	2.04a	6.4a	25a	3.9a
	90	2020a	12.6a	1.92b	6.5a	25a	3.8a
	0	8800b	-	-	-	113b	16.4b
YEAR	45	9010b	-	-	-	118b	19.7a
	90	10230a	-	-	-	132a	20.8a

#Rate of S applied from November 1983 to March 1984.
*Means within columns within subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

The N:S ratios obtained in the Sward II are of the same magnitude as those described in Experiment No. 1 and those presented by Mitchell (1980) for Pensacola bahiagrass. Significant decreases in the N:S ratios of forages collected in three out of four harvests were a consequence of the S accumulation in the forage tissues.

Significant increases in N concentration and average weight of petioles + leaflets were observed in white clover sampled from the sward at the time of the first harvest, due to the S fertilization (Table 3-8). Later in the season (June), white clover plants were stunted, and did not show significant responses.

Sulfur concentrations were significantly higher, and N:S ratios were significantly lower in plants growing in plots fertilized with S, in both May and June. It should be pointed out that S concentrations and N:S ratios of the legume fell in the range considered to be adequate for normal plant development (Andrew, 1977; McNaught and During, 1970; Metson, 1973). Apparently those few white clover plants that survived in the stand could get small but necessary amounts of S through sources other than the fertilizers (e.g., readily-available in the soil, mineralized from organic matter, or atmospheric S). Some significant changes in Ca, Mg, and P concentrations in the legume were observed, but they do not seem to have major consequences for the nutrition of those plants. Once again, K concentrations in white clover was significantly lowered

where S fertilizer was applied. Values of the micronutrients (Cu, Fe, Mn, and Zn) from the legume samples showed Cu concentrations being close to the deficiency level (McNaught, 1970; Martin and Matocha, 1973; Tengku Maamun, 1985).

Table 3-8. Average (petiole + leaflets) dry weight, macronutrient and micronutrient concentrations, and N:S ratio in the white clover sampled in the Sward II in May and in June 1984.

Response variables	S rates (kg ha^{-1})					
	0	45#	90	0	45	90
	May 1984			June 1984		
Weight (mg)@	43.1c*	47.8b	55.6a	23.5a	23.6a	23.3a
N (g kg^{-1})	30.6b	33.6a	30.0b	30.7a	31.4a	31.7a
S (g kg^{-1})	1.70b	2.11a	1.65b	2.03b	2.24a	2.05b
N:S (ratio)	18.0a	15.9b	18.2a	15.1a	14.ab	15.5a
Ca (g kg^{-1})	13.4b	12.7b	16.8a	17.1a	16.4a	17.6a
Mg (g kg^{-1})	4.6b	4.6b	5.2a	4.8b	4.8b	5.2a
K (g kg^{-1})	36.1a	30.7b	18.0c	29.2a	27.2a	22.2b
P (g kg^{-1})	3.2a	2.6b	3.2a	3.0a	3.4a	3.2a
Cu (mg kg^{-1})	4.1a	3.5b	3.9ab	4.4a	3.7b	4.2a
Fe (mg kg^{-1})	57a	58a	53b	66a	71a	66a
Mn (mg kg^{-1})	42a	32a	35a	38a	31a	34a
Zn (mg kg^{-1})	19a	19a	19a	21a	20a	22a

#Rate of S applied from November 1983 to March 1984.
@Average weight of each petiole with three leaflets.
*Means within rows followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Summary

Two sets of experimental plots allocated in a mixed sward of white clover and Pensacola bahiagrass established in 1952 on a virgin Spodosol were used in the present studies. One set of plots was established in 1952 for a lime-micronutrient experiment (Sward I), and the other in

1963 for a rototilling-lime source-N rate study (Sward II). White clover showed poor growth in both set of plots, in recent years. Studies were carried out to investigate the possible effects of S fertilizations on those plots. Fertilizer was applied with or without S to Sward I in 1980, 1981, and 1983/84. Where applied, the total S rate was 200 kg ha^{-1} during the 4-year experimental period. Significant increases in forage yield, and N and S contents due to S fertilization were observed every year during the 4 years of the experiment. Sulfur concentration in the forages was higher where S was applied, especially in the first and in the fourth year. When the number of white clover plants in the sward was appreciable, S fertilization resulted in significant increase in N concentration in the forages. Sulfur concentration and N:S ratio were found in the S-deficient range in white clover tissues. The N:S ratio of Pensacola bahiagrass ranged from 3.5:1 to 8.1:1, which indicated a low N supply for the grass. The apparent S recovery from the fertilizer was 13.2% over the 4-year period. Plots receiving S fertilizer had lower total N in the soil, whereas the total N:total S in the soil was 7.5:1 irrespective of S applications. Stolon-root tissue of Pensacola bahiagrass contained 31 to 33 kg S ha^{-1}, and had a N:S ratio of 4.1:1. There was no evidence of S storage in the stolon-root tissue of the grass during the spring season. In Sward II, S was applied at 0, 45 and 90 kg ha^{-1} in 1983/84. Significant increases in forage yield, N and S

concentrations, and N and S contents in the mixed forages were observed. The apparent S recoveries from the fertilizer were 7.3 and 4.8% for the 45 and 90 kg S ha^{-1} applications, respectively. In the spring of 1984, severe frost caused drastic damage to the plants in both swards. The limited number of surviving white clover had apparently adequate S concentration and N:S ratio. Lower K concentrations in white clover and stolon-root tissues of Pensacola bahiagrass, as well as lower Mehlich I-extractable K in the soil were obtained in the S-treated plots than in the control.

CHAPTER IV
EFFECTS OF SULFUR AND MOLYBDENUM APPLICATIONS ON WHITE CLOVER ALONE AND MIXED WITH PENSACOLA BAHIAGRASS

Introduction

An adequate nutrient supply for the legume is one of the major considerations for the productivity and maintenance of a mixed pasture.

For 5 years before this study, white clover was not highly productive in a long-term experiment where liming and fertilizer P and K applications were included in management practices for a mixed sward on a Florida Spodosol.

Inadequate S supply has been a severe limitation for plant growth, N_2-fixation, and/or N metabolism in white clover (Andrew, 1977; McLaughlin and Holford, 1982; Robson, 1978). White clover productivity and maintenance in Florida Spodosols have been found to depend on S fertilization (Harris et al., 1968; Tengku Maamun, 1985). Mixed white clover and grasses have also been very responsive to S additions on the Spodosol (Neller et al., 1951b; Neller and Bartlett, 1959).

Mitchell and Blue (1981) reported that phosphate-extractable S averaged only 2 to 4 mg S kg^{-1} of soil in the A horizon and 1 to 2 mg S kg^{-1} of soil in the E horizon of

Florida Spodosols. It should be emphasized that those soil horizons support the most active roots of white clover.

Molybdenum deficiency in forage legumes has been observed in many areas of the world (Gupta and Lipsett, 1981). Although the importance of this micronutrient in the N_2-fixation process has long been known (Anderson, 1956), the requirement for Mo by forage legumes in the southeastern United States has received little attention (Fales and Thompson, 1982). Upon sulfate application, Mo uptake by the plants may be depressed as a consequence of the competition between the two anions (SO_4^{2-} and MoO_4^{2-}) for absorption sites on the roots (Gupta and Munro, 1969; Reddy et al., 1981; Reisenauer, 1963; Singh and Kumar, 1979).

The principal objective of this investigation was to evaluate the responses of white clover in the greenhouse and white clover-Pensacola bahiagrass in the field to S and Mo applications on a Spodosol.

Materials and Methods

Greenhouse Experiment

Soil samples (0 to 15 cm) were collected in November 1982 from limed plots which received micronutrients from 1952 to 1959. Those plots were located in the Sward I. Molybdenum at 1.1 kg ha^{-1} was applied in 1952 as sodium molybdate. Soil from subplots with and without previous S fertilization was collected separately. Samples were air dried and sieved through a 2-mm screen. Selected chemical

properties are presented in Table 4-1. Soils were limed with 500 mg dolomitic lime kg^{-1} of soil. Two kilograms of limed soil were than placed above 1 kg of acid-washed quartz gravel in plastic pots.

Table 4-1. Some chemical properties of the soil prior to the greenhouse experiment.

Soil properties	Previous S fertilization, mg kg^{-1}	
	0	55#
pH (water, 2:1)	5.5	5.4
pH (1 M KCl, 2:1)	4.9	4.8
Organic C (g kg^{-1})	25	25
Total S (mg kg^{-1})	159	171
Extractable S (mg kg^{-1})	3.0	3.3
Extractable Ca (cmol $[p^+]$ kg^{-1})	6.6	6.4
Extractable Mg (cmol $[p^+]$ kg^{-1})	0.96	0.77
Extractable K (cmol $[p^+]$ kg^{-1})	0.14	0.10

#Total rate of S applied in the field during 1980 and 1981.

The greenhouse experiment was a 2x3x4 factorial, with three replications in a randomized complete block design. Treatments included soils with and without previous S fertilization, three current S rates (0, 15, and 30 mg kg^{-1} of soil), and four rates of Mo (0, 42, 84, and 126 µg Mo kg^{-1} of soil). Soil with previous S fertilization received 40 kg S ha^{-1} in November 1980, 30 kg S ha^{-1} in March 1981, and 40 kg S ha^{-1} in November 1981. Current S was applied as gypsum ($CaSO_4.2H_2O$), and Mo was supplied as $Na_2MoO_4.2H_2O$. Dilute solutions of analytical-grade KH_2PO_4, KCl, and H_3BO_3 were added to each pot to provide 20 mg P, 75 mg K, and 25 µg B kg^{-1} of soil, respectively.

The white clover 'Osceola' was grown in the pots. Twenty five seeds were planted, and were immediately inoculated by adding 20 mL of a suspension containing Rhizobium trifolii (15 g inoculant L^{-1} of water). Plants were thinned to six per pot, and plants in all pots were harvested 75 days after sowing. Plant tops (leaflets + petioles + stolons) were separated from the roots. After removing the roots, the soil was air dried and passed through a 2-mm screen. Plant material was dried at 70°C and weighed.

Field Experiment

Plots that had produced uniform herbage yields and with similar previous fertilization were selected from the Sward I experiment. Each plot had been divided into two subplots, with and without S fertilization. Four SxMo (2x2 factorial) combinations were applied to all subplots. Each subplot was 20.5 m^2 and the treatments were allocated in randomized complete block design with four replications. Current S rates were 0 and 30 kg ha^{-1}, as gypsum, whereas Mo levels were 0 and 0.2 kg ha^{-1}, as sodium molybdate. All 32 subplots had been limed (1.1 Mg dolomitic lime ha^{-1}) in November 1982, and received 40 kg P ha^{-1}, as concentrated superphosphate, and 140 kg K ha^{-1}, as potassium chloride. Sulfur, Mo, P, and K were applied by hand in December 1982. The experimental area had been reseeded with white clover 'Osceola' 20 days before fertilization.

The forage was harvested four times (May, late June, August, and October) in 1983. At each harvest, yield was obtained and forage samples were collected. In May 1983 before harvesting, pure white clover samples were hand-plucked in all subplots. Soil samples (0 to 15 cm) were collected in October 1983, after the fourth harvest.

Plant and Soil Analyses

All plant tops were ground to pass a 0.85-mm stainless-steel screen in a Wiley mill. Total N was determined by a semimicro-Kjeldahl procedure with salicylic acid and sodium thiosulfate to include nitrate and nitrite in the determination. For mineral element analysis, the plant tissue was dry ashed. The ash was digested with 20 mL of 6 M HCl, followed by evaporation to dryness. The residue was dissolved in 2.25 mL of 6 M HCl. The solution was heated, received about 10 to 15 mL of deionized water,and was filtered. The filter paper was washed several times with deionized water, and the filtrate made to 50 mL volume. One gram of plant tissue was ashed for Ca, Mg, and K analyses, whereas 2 g were used for Mo analysis. Calcium and Mg in the extracts were determined by atomic absorption spectrometry. For Mo determination, the extracts were treated with dithiol solution, and the chelated Mo was extracted into isoamyl acetate (Bingley, 1963). The Mo complex was introduced into a graphite furnace, and Mo was analyzed by atomic absorption spectrometry (Khan et al., 1979).

Total S determinations in plant tissue and soil were done by direct combustion of the sample under an O_2 stream in a Leco SC-132 Sulfur Determinator with an infrared detector (Leco Corporation, 1980).

Soil pH in water and in 1 M KCl were determined in 2:1 (w/v) suspensions. Soil samples were extracted with Mehlich's double-acid solution (Nelson et al., 1953) for Ca, Mg, and K determinations. Organic C was determined by the Walkley-Black procedure as modified by Walkley (1947).

Soil extractable sulfate-S was determined turbidimetrically (Butters and Chenery, 1959 and Massuomi and Cornfield 1963), after extraction with a 500 mg P L^{-1} Ca $(H_2PO_4)_2.2H_2O$ solution (Fox et al., 1964).

Statistical Analyses

Analysis of variance, means comparisons by using Duncan's Multiple Range Test - DMRT, and regression analysis were accomplished through SAS programs described by Freund and Littell (1981). The correlation procedure was described in SAS (1982).

Results and Discussion

Greenhouse Experiment

The effects of all treatments on herbage yield, N and S concentrations, N and S contents, and N:S ratio in white clover are presented in Tables 4-2 and 4-3. Previously applied S in the field during 1980 and 1981 did not have any

significant effect on these variables. However, significant increases in all except N:S ratio were obtained from current S fertilization. The results agreed with the findings by Andrew (1977), Harris et al. (1968), and Tengku Maamun (1985).

Table 4-2. Effects of previous and current S fertilizations on forage yield, N and S concentrations, N:S ratio, and N and S contents in white clover (plant top) grown in the greenhouse.

S rates	Forage					
	Yield	N	S	N:S	N	S
mg kg^{-1}	g pot^{-1}	----g kg^{-1}----		ratio	---mg pot^{-1}---	
		Previous S fertilization				
0	7.73a*	23.4a	1.14a	20.8a	187a	9.1a
55#	8.45a	23.6a	1.11a	21.6a	204a	9.5a
		Current S fertilization				
0	4.37c	20.9c	0.99c	21.3ab	91c	4.3c
15	9.32b	23.5b	1.08b	21.9a	219b	10.0b
30	10.58a	26.2a	1.30a	20.3b	277a	13.7a

#Total rate of S applied in the field during 1980 and 1981.
*Means within columns within subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Table 4-3. Effects of Mo fertilization of forage yield, N and Mo concentrations, and Mo contents in white clover (plant top) grown in the greenhouse.

Mo rates	Forage			
	Yield	N	Mo	Mo
µg kg^{-1}	g pot^{-1}	g kg^{-1}	mg kg^{-1}	µg pot^{-1}
0	7.73a*	23.2a	0.4d	1.6d
42	8.35a	23.7a	7.2c	55.4c
84	8.31a	23.3a	13.1b	97.7b
126	7.98a	23.9a	17.8a	133.4a

*Means within columns followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Regression analysis showed that forage yield (g pot^{-1}), and N and S contents (in mg pot^{-1}) were related to S rate (mg kg^{-1} of soil) according to the following equations:

Yield = 4.37 + 0.455 S - 0.008 S^2 (R^2= 0.88)

N content = 90.98 + 10.856 S - 0.155 S^2 (R^2= 0.91)

S content = 4.28 + 0.449 S - 0.004 S^2 (R^2= 0.93)

Plants growing in pots that did not receive S produced only 41% of the maximum forage yield (Fig. 4-1), had smaller numbers of petioles per pot, showed weak stolon development, and did not flower until harvest time. Retardation of flowering time in a S-deficient legume (Stylosanthes humilis H. B. K.) has been reported by Robinson and Jones (1972).

Fig. 4-1. Sulfur effect on white clover growth (left to right = increasing current S rates at constant previous S and Mo fertilizations).

Sulfur concentration increases in clover that received S fertilizers were described by Andrew (1977) and Thompson and Neller (1963). Sulfur concentrations obtained in this experiment (0.99 to 1.30 g S kg^{-1}) were in the same range as those presented by Andrew (1977) for S-deficient plants. These values were much lower than the values accepted as the critical concentration of this nutrient in white clover (Andrew, 1977; McNaught and During, 1970; Metson, 1973).

Significant beneficial effects were observed for both N concentration and content of white clover from current S applications. Similar effects were also reported by Andrew (1977), Tengku Maamun (1985), and Spencer (1959). As noted for S, N concentration in white clover was lower than that obtained elsewhere (Andrew, 1977; Harris et al., 1968). In terms of concentrations and contents in the legume, N and S were highly correlated (0.79*** and 0.97***, respectively). The level of plant S required for maximum N concentrations in tops of legumes is higher than that for maximum yields (Robson, 1978). Since the maximum S applied to the Spodosol was not enough to bring herbage S concentration to desired levels, it is possible that higher S rates would have increased herbage N concentration.

After the work by Dijkshoorn et al. (1960), the N:S ratio has been suggested as another valuable means of diagnosis of S deficiency. Ratios between 16:1 to 19:1 have been considered adequate for legumes. Wider ratios, like

those obtained in this study, are indicative of S inadequacy for white clover.

Molybdenum application had no significant effect either on herbage yield or N concentration (Table 4-3). Responses of legumes to Mo have been reported to be affected by lime (Anderson, 1956; Gupta and Lipsett, 1981). Hawes et al. (1976) applied Mo rates to an Ultisol with pH 6.4 and found no beneficial effect on clover yield. Peaslee (1982) failed to obtain significant responses of forage legumes to Mo application in limed soils, especially at pH values higher than 5.5 in the southern United States. The soil used in the present study had been limed periodically, had a pH in water of 5.5, and received 500 mg dolomitic limestone kg^{-1} of soil at the beginning of the pot experiment. Another soil factor that might have contributed to the lack of response to Mo was the relatively high organic matter content (about 43 g kg^{-1}), which may have adsorbed at least part of the applied Mo (Karimian and Cox, 1978).

Concentrations and contents of Mo in the forage were linearly increased by Mo applications, as expressed by the following equations:

$$\text{Mo concentration} = 1.097 + 0.138\ \text{Mo} \qquad (R^2 = 0.66)$$

$$\text{Mo content} = 7.649 + 1.032\ \text{Mo} \qquad (R^2 = 0.65)$$

where Mo concentration and content were expressed in $\mu g\ kg^{-1}$ and $\mu g\ pot^{-1}$, respectively, and Mo represented Mo rate in $\mu g\ kg^{-1}$ of soil.

When Mo was supplied at rates of 84 or 126 μg kg^{-1} of soil, Mo concentrations in the plants ranged from 10 to 22 mg kg^{-1}. White clover plants had Cu concentrations of 5 mg kg^{-1}. The relatively high Mo concentrations associated with such Cu concentrations in the same plants, might result in Mo toxicosis-Cu deficiency in animals (they are difficult to differentiate in animals) if they were fed exclusively with such forage (Ward, 1978). As suggested by Johnson (1966), Mo concentrations in this range did not cause any toxicity to the legume.

The S x Mo interaction was significant at $P<0.10$ for the Mo concentration in the legume (Table 4-4).

Table 4-4. Effects of S and Mo interaction@ on Mo concentrations in the white clover grown in the greenhouse.

Current S rates	Mo rates, μg kg^{-1}			
	0	42	84	126
mg kg^{-1}	Oven-dry forage Mo, mg kg^{-1}			
0	1.3a*	9.7a	18.3a	22.7a
15	0.2b	6.4ab	11.1b	16.2b
30	0.1b#	5.6b	10.7b	14.6b

@Interaction significant at the 10% level of probability.
#The average Mo concentration at S=30 and Mo=0 was 0.14 mg kg^{-1}.
*Means within columns followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Sulfur fertilization resulted in significant decreases in Mo concentration in the plant tops, as previously reported by Gupta and Munro (1969), Reddy et al. (1981), and

Reisenauer (1963), Singh and Kumar (1979). Without Mo application, the highest S rate reduced the Mo concentration in the forage to 0.14 mg kg^{-1}, which is considered a marginal value for Mo concentration in clover (Cheng and Ouellette, 1973).

Plant concentrations and contents of Ca, Mg, and K, as well as the ratios between pairs of these nutrients, are shown in Table 4-5. Although some changes in Ca and Mg concentrations due to S fertilization were statistically significant, they varied only slightly, and the concentrations always exceeded the critical concentration for the legume (Martin and Matocha, 1973). Total uptake of Ca and Mg were significantly increased by the current application of gypsum, and the ratio between these two nutrients in the plant tissue was not significantly changed. Potassium concentration and content, and its ratio with Ca or Mg were significantly altered by either previous or current S fertilization. Both K concentration and uptake decreased in the plant tissue as the rate of S was increased. The lowest K concentrations (about 10 g kg^{-1}) fell in a range from 8 to 15 g kg^{-1} considered to be critical for K deficiency for annual clovers (Martin and Matocha, 1973). Lower K concentration in clover that received S fertilization than in treatments without S were also shown by Bledsoe and Blaser (1947) and Harris et al. (1968).

Soil pH (in water or in 1 M KCl), total and extractable S, and Mehlich-I extractable Ca, Mg, and K determined at the

end of the experiment are presented in Table 4-6. Since Mo had no significant effect on any of these variables, only the effects of S are presented and discussed. All the means presented in the subtable concerning previous S fertilization are the average values over the current S and Mo fertilizations, and the reader should not conclude, for example, that total and extractable S increased in soil with no previous S application, during the greenhouse experiment. Application of gypsum to soil in the pots resulted in significant reduction of pH values. The same acidifying influence of gypsum in the soil was not observed as a residual effect of the previous S applications in the field. This suggested that most of the SO_4^{2-} had been leached out of the soil before the soil was collected for the greenhouse study.

Current S applications resulted in marked changes in the total and extractable S in the soil. From the plant nutrition standpoint, increases in extractable S were of major significance, and this variable was positively correlated with the white clover S uptake (r=0.90***). Extractable S (mg kg^{-1}) was also related to the S rate (mg kg^{-1}) applied to the soil according to the equation:

$$\text{Extractable S} = 3.456 + 0.127\ S + 0.011\ S^2 \quad (R^2 = 0.97),$$

Extractable Ca and Mg decreased as the rate of currently applied S increased, and these changes can be attributed to increased Ca and Mg absorption by the plants at higher S rates.

Table 4-5. Effects of previous and current S fertilizations on some macronutrient concentrations, contents, and ratios in white clover grown in the greenhouse.

S rates	Forage								
	Ca	Mg	K	Ca	Mg	K	Ca:Mg	Ca:K	Mg:K
mg kg^{-1}	--------g kg^{-1}--------			-----mg pot^{-1}------			--------ratio--------		
				Previous S fertilization					
0	15.5a*	4.2a	17.4a	119a	32a	115a	3.7a	1.1b	0.29b
55#	14.6a	3.8b	13.9b	122a	32a	97b	3.8a	1.3a	0.34a
				Current S fertilization					
0	15.7a	4.2a	26.2a	69c	19c	115a	3.7a	0.6c	0.16c
15	14.5b	3.9a	10.9b	135b	36b	100b	3.7a	1.4b	0.37b
30	14.9b	3.9a	9.9b	158a	42a	103b	3.8a	1.6a	0.41a

#Total rate of S applied in the field during 1980 and 1981.
*Means within columns within subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Extractable K was lower in the soil that previously received S application either before or after the greenhouse experiment (Tables 4-1 and 4-6). Current gypsum fertilization resulted in significant decreases in extractable soil K. Nutrients were not leached out of the pots during the experiment. Tengku Maamun (1985) showed that the soil which received S application had only one third as much extractable K as the soil that did not receive treatment. The low extractable soil K together with low plant K concentrations can not be explained.

Table 4-6. Soil pH (2:1 in water or 1 M KCl), soil total and extractable S, and Mehlich-I extractable Ca, Mg, and K in the Spodosol used in the greenhouse experiment.

S rates	pH		S		Mehlich-I Extractable		
	H_2O	KCl	Total	Ext.@	Ca	Mg	K
mg kg^{-1}			---mg kg^{-1}---		---cmol(p^+) kg^{-1}---		
			Previous S fertilization				
0	5.4a*	5.0a	163a	9.3a	6.5a	1.1a	0.08a
55#	5.4a	5.0a	161b	9.6a	6.4a	0.8b	0.06b
			Current S fertilization				
0	5.6a	5.1a	157c	3.5c	6.7a	1.1a	0.08a
15	5.3b	4.9b	162b	7.8b	6.3b	0.9b	0.06b
30	5.3b	4.9b	167a	15.1a	6.4ab	0.9b	0.06b

@Stands for phosphate-extractable S.
#Total rate of S applied in the field during 1980 and 1981.
*Means within columns within subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Field Experiment

The effects of S treatments on total yield and contents of total N and S in the white clover + Pensacola bahiagrass forage from four harvests in 1983 are presented on Table 4-7.

Table 4-7. Effects of previous and current S fertilizations, and Mo fertilizations on forage yield, and N and S contents of white clover + Pensacola bahiagrass grown in the field. Total of 4 harvests in 1983.

Nutrient rates	Forage		
	Yield	N	S
kg ha^{-1}	----------kg ha^{-1}----------		
	Previous S fertilization		
0	11400b*	167b	20.1b
110#	12470a	181a	23.0a
	Current S fertilization		
0	11520a	164a	20.4a
30	12350a	185a	22.8a
	Mo fertilization		
0	11660a	168a	21.7a
0.2	12200a	180a	21.4a

#Total rate of S applied during 1980 and 1981.
*Means within columns within subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

The grass component of the sward was largely responsible for the magnitude of these values, since it was dominant in the mixture. Statistically significant increases in these three variables occurred only in the plots that received S previously. Pensacola bahiagrass with a deeper root system than white clover would take up more S from that previously

applied because S was generally present in deeper soil layers (see data in Chapter VI). The higher S uptake in the forages should have resulted in better N assimilation, and the forages increased yield. From the current S fertilization only 8% was recovered in the above-ground forage. This suggested a high loss (mainly by leaching) of SO_4 from the soil. In fact, between the current S application and the last forage harvest, about 1330 mm of rain fell on the experimental area. This might have been a cause for S leaching (McKell and Williams, 1960; Shock et al., 1983).

Table 4-8. Effects of previous and current S fertilizations on forage yield, N and S concentrations, N:S ratio, and N and S contents in white clover + Pensacola bahiagrass harvested in May 1983.

S rates	Forage (legume + grass)					
	Yield	N	S	N:S	N	S
kg ha^{-1}	kg ha^{-1}	----g kg^{-1}----		ratio	--kg ha^{-1}--	
			Previous S fertilization			
0	2860b*	19.7a	1.45a	14.1a	57a	4.1b
110#	3150a	19.1a	1.47a	13.5a	60a	4.6a
			Current S fertilization			
0	2860a	18.7b	1.42a	13.9a	54b	4.0a
30	3150a	20.1a	1.49a	13.8a	63a	4.7a

#Total rate of S applied during 1980 and 1981.
*Means within columns within subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Data obtained at the first forage harvest in May (Table 4-8) showed significant increases in forage yield and S content as a result of the previous S application. Grass alone probably was contributing to these increases since

previous S fertilization did not affect the yield of white clover in the greenhouse experiment (Table 4-9) nor its N and S concentrations in the field (Table 4-9). Current S application significantly increased N concentration and content in the mixed sward, and N concentration in the legume component of the sward at the first harvest. These benefits can be attributed to improvements in the N_2-fixation system and in the utilization of N fixed in the forage legume (Andrew, 1977; Robson, 1978; Shock et al., 1983). Nitrogen and S concentrations in white clover tissues were highly correlated (r= 0.91***).

Table 4-9. Effects of previous and current S fertilizations, and Mo fertilizations on N and S concentrations, N:S ratio, and Mo concentration in white clover sampled in the mixed Sward in May 1983.

Nutrient rates	Forage (only legume)			
	N	S	N:S	Mo
kg ha^{-1}	------g kg^{-1}------		ratio	mg kg^{-1}
		Previous S fertilization		
0	29.9a*	1.32a	22.7a	3.0a
110#	29.8a	1.34a	22.4a	2.9a
		Current S fertilization		
0	28.9b	1.28b	22.7a	3.4a
30	30.8a	1.38a	22.4a	2.5b
		Mo fertilization		
0	29.5a	1.30a	23.0a	0.5b
0.2	30.3a	1.36a	22.1a	5.3a

#Total rate of S applied during 1980 and 1981.
*Means within columns within subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Current gypsum addition increased the S concentration but reduced the Mo concentration in white clover. Similar results were obtained in the greenhouse study. Also, Mo fertilization was responsible for a significant increase in Mo concentration of the legume. However, even the range of the Mo concentration was lower than that obtained for white clover in the greenhouse. The open system for leaching in the field may have resulted in some movement of the MoO_4^{2-} out of the clover roots zone.

At the time of the fourth harvest in October, grass essentially dominated the sward. Only previous S applications resulted in significant increases in yield, and in N and S contents of the forage (Table 4-10).

Table 4-10. Effects of previous and current S fertilizations on forage yield, and N and S contents in the sward harvested in October 1983.

S rates	Forage (essentially grass)					
	Yield	N	S	N:S	N	S
kg ha^{-1}	kg ha^{-1}	----g kg^{-1}----		ratio	--kg ha^{-1}--	
			Previous S fertilization			
0	2750b*	11.8a	1.98a	6.0a	32b	5.4b
110#	3200a	11.7a	2.01a	5.9a	38a	6.5a
			Current S fertilization			
0	2920a	11.8a	1.99a	6.0a	34a	5.8a
30	3040a	11.7a	1.99a	5.9a	36a	6.0a

#Total rate of S applied during 1980 and 1981.
*Means within columns within subtables followed by the same letters are not significantly different ata the 5% level of probability (DMRT).

The N:S ratio of the sward did not change with S fertilization (Table 4-10). The values (about 6:1) were

lower than those suggested as critical for grasses (13.7:1) by Dijkshoorn et al. (1960). On the other hand, when pure white clover from the mixed sward was sampled, the N:S ratio (about 22 to 23:1) was wider than the 17.5:1 suggested as the critical value for legumes by the same author. In addition, S concentrations in pure white clover were below the critical concentration for this legume (Metson, 1973). Therefore, a legume plant, well supplied with N through biological N_2-fixation, had an excessive N:S ratio because of S limitation, even in the S-fertilized plots. As legume development was still impaired, the stand of white clover was not adequate to supply all the necessary N for the grass late in the year. Consequently, Pensacola bahiagrass that could better absorb S from the soil had a limited N supply, and showed narrow N:S ratios. Similar behaviours of grasses and legumes in relation to these nutrients were described by Adams (1973), Anderson and Spencer (1950), and Jones and Martin (1964).

Analysis of soil samples collected after the fourth forage harvest showed no significant ($P<0.05$) changes in soil pH, and in Mehlich-I extractable Ca and Mg with either previous or current S applications.Soil pH in water averaged about 6.0, whereas extractable Ca and Mg had mean concentrations of 5.60 and 0.97 cmol (p^+) kg^{-1} of soil, respectively. However, Mehlich-I extractable K was significantly decreased (0.09 vs 0.07 cmol [p^+] kg^{-1}) as a consequence of the current S fertilization. A similar effect of

current S fertilization on extractable K was observed in the greenhouse experiment.

Summary

Greenhouse and field experiments were carried out with a Florida Spodosol that had supported a mixed sward of white clover and Pensacola bahiagrass for 30 years.

Soils from plots with or without previous S application were used in a pot experiment. Rates of 0, 15, and 30 mg S kg^{-1} of soil, and rates of 0, 42, 84, and 126 µg Mo kg^{-1} of soil were tested. White clover was grown. Previous S fertilization did not have marked effects on white clover. Current S application resulted in significant increases in forage yield, N and S concentrations, and N, S, Ca, and Mg contents. As the current S rate was increased, plants had longer stolons, larger number of petioles and leaves, and more flowers at harvest time. However, significant decreases in the N:S ratio, and in the K and Mo concentrations and contents were observed. Even at the highest S rate, S concentration, and N:S ratio in the legume were not considered adequate for white clover. Molybdenum concentration and content in the legume were linearly increased by Mo fertilization. Current S addition decreased soil pH, and extractable K while total and extractable S were increased. Extractable S in the soil was closely related to the legume S content (r= 0.90***).

In the field experiment, rates of 0 and 30 kg S ha^{-1}, and 0 and 200 g Mo ha^{-1} were applied to the mixed sward. Both subplots with or without previous S application were fertilized. Previous S fertilization increased forage yield, and N and S contents in the mixed sward. Current S fertilization increased N and S concentrations, and decreased Mo concentration in white clover. The N:S ratio in white clover was wider and in the grass was more narrow than the critical values proposed by Dijkshoorn et al. (1960). These results suggested that in the experiment conducted, the lack of S was still limiting normal development of white clover. The white clover, in turn, could not supply enough N to the grass in the subsequent season for growth rates similar to those observed during the previous 25+ years.

CHAPTER V
EFFECTS OF TWO SOURCES OF SULFUR COMBINED WITH POTASSIUM ON WHITE CLOVER GROWN IN A GREENHOUSE

Introduction

Sulfur fertilization was found to be necessary for normal white clover (Trifolium repens L.) development on a Florida Haplaquod (Harris et al., 1968, Tengku Maamun, 1985). A previous experiment (reported in Chapter IV) demonstrated that white clover fertilized with S up to 30 mg kg^{-1} of soil did not reach an adequate S concentration in the plant tissue and had an excessively high N:S ratio. It was also shown that K application at 75 mg kg^{-1} soil was not sufficient for normal legume growth.

When gypsum was applied to the soil used in the greenhouse experiment (Chapter IV), K concentration in white clover as well as extractable K in the soil decreased as S rates increased. Similar results were obtained from the field experiment (Chapter III). White clover plants and the stolon-root tissue of Pensacola bahiagrass (Paspalum notatum Flugge) had significantly lower K concentrations from soil that received S as K_2SO_4. Lower Mehlich-I extractable K was also noted in these soil samples.

Tengku Maamun (1985) used the same Myakka fine sand in a greenhouse experiment, and showed statistically lower K concentration in white clover and lower extractable K in soil where S was added. Sulfur was supplied as K_2SO_4, and K as K_2SO_4 + KCl + KH_2PO_4.

The objectives of this experiment were: a) to test the effect of S application at higher rates than in the previous experiments; b) to compare gypsum and potassium sulfate as the S-supplying source; c) to gain information on S distribution in parts of white clover plants; and d) to obtain more data with white clover in the light of the previously suggested S and K relationship.

Materials and Methods

Soil samples were collected from the 0 to 15 cm depth in October 1984 from treatment No. 4 in the original long-term experiment designated as Sward I. Separate samples were taken from subplots with and without previous S applications during the last 4 years. Air-dried samples were passed through a 2-mm screen, and 2 kg portions were weighed into plastic bags. Analyses are presented in Table 5-1.

Five S treatments (control, and 22.5 and 45 mg S kg^{-1} of soil supplied as reagent-grade gypsum or potassium sulfate), two current K rates (110 and 220 mg kg^{-1} of soil), and soils with or without previous S fertilization were studied. Those 20 treatments (5x2x2) were arranged in a randomized complete block design, with four replications.

Both current S and current K were mixed in solid form with the soil, by hand-shaking, before the soil was placed into plastic pots. Each pot had 1 kg of acid-washed quartz gravel in the bottom.

Table 5-1. Some chemical properties of the soil prior to the pot experiment.

Soil properties	Previous S fertilization	
	No	Yes#
pH (water, 2:1)	5.8	5.9
pH (1 M KCl, 2:1)	5.3	5.4
Organic C (g kg^{-1})	22.8	25.0
Total N (mg kg^{-1})	1221	1333
Total S (mg kg^{-1})	161	171
Organic C: total N	18.7	18.8
Organic C: total S	142	146
Total N: total S	7.6	7.8
Extractable Ca (cmol[p^+]kg^{-1})	6.15	6.80
Extractable Mg (cmol[p^+]kg^{-1})	1.05	1.10
Extractable K (cmol[p^+]kg^{-1})	0.09	0.06
Extractable P (mg kg^{-1})	39	39
Extractable S (mg kg^{-1})	4.0	4.2

#Total amount of S applied in the field experiment in 1980, 1981, and 1983/84 was 200 kg ha^{-1}.

Twenty-five seeds of white clover 'Osceola' were planted in each pot, and inoculated with 20 mL of a suspension (15 g inoculant L^{-1} of water) containing Rhizobium trifolii. Solutions containing reagent grade $NaH_2PO_4.H_2O$, H_3BO_3, $CuCl_2.2H_2O$, and $Na_2MoO_4.2H_2O$ were applied to all pots in amounts equivalent to 20 mg P, 0.25 mg B, 1 mg Cu, and 0.1 mg Mo kg^{-1} of soil. Plants were thinned to six per pot. The first harvest was done 68 days after sowing. Only developed petioles + leaflets were removed from the plants.

Forty-eight days later, during the second harvest, tissues of petioles + leaflets, stolons, and roots were collected separately from each pot. At each harvest time, petioles + leaflets, and flower heads were counted in each pot. All plant materials were dried at 70°C, weighed, and ground to pass a 0.85 mm stainless-steel screen in a Wiley mill. Petiole + leaflet, stolon, and root samples were analyzed for N and S, whereas petioles + leaflets were further analyzed for Ca, Mg, K, and P.

Soil from the pots was air-dried, passed through a 2-mm screen, and split several times to give about 100 g samples.

Organic C, total N, total and extractable S, and extractable Ca, Mg, K, and P as well as soil pH in water and KCl were determined. Laboratory procedures used for both soil and plant analysis were described in Chapter III, experiment No. 1.

All statistical analyses were done according to procedures described by Freund and Littell (1981) and SAS (1982). The number of flower heads was logarithmically transformed prior to analysis of variance.

Results and Discussion

The main effects of the treatments on herbage yield and N and S measurements in petioles + leaflets of white clover in the first harvest, and in the whole legume plant in the second harvest are presented in Tables 5-2 and 5-3, respectively. As was found in a previous experiment with the

same soil (Chapter IV), previous S fertilization had no significant effect on any of these variables. Current high K application resulted in significant increases of S concentration and content, and decrease of N:S ratio in the legume

Table 5-2. Main effects of previous S fertilization, and current K and S fertilizations on forage yield, N and S concentrations, N:S ratio, and N and S contents of white clover at the first harvest.

Main effect	Forage (petioles + leaflets)					
	Yield	N	S	N:S	N	S
	g pot^{-1}	----g kg^{-1}----		ratio	--mg pot^{-1}--	
			Previous S fertilization			
No	7.32a*	29.6a	1.69a	18.2a	231a	13.5a
Yes#	7.27a	29.5a	1.65a	18.1a	227a	12.9a
			Current K fertilization			
Low (110)@	7.19a	29.7a	1.64b	18.6a	227a	12.8b
High (220)	7.39a	29.4a	1.70a	17.8b	231a	13.6a
			Current S fertilization			
Control(0)	2.37d	18.7b	0.87c	21.6a	44d	2.1d
Gypsum (22.5)	8.25c	31.8a	1.81b	17.7b	262c	14.9c
Gypsum (45)	8.82a	32.6a	2.00a	16.3c	287a	17.7a
K_2SO_4 (22.5)	8.37bc	32.0a	1.80b	18.0b	269bc	15.1c
K_2SO_4 (45)	8.66ab	32.6a	1.88b	17.4b	282ab	16.3b

#Total amount of S applied in the field experiment in 1980, 1981, and 1983/84 was 200 kg ha^{-1}.
@Nutrient applied in the current fertilization, in mg kg^{-1} of soil.
*Means within columns in subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Table 5-3. Main effects of previous S fertilization, and current K and S fertilizations on forage yield, and N and S contents of whole plants of white clover at the second harvest.

Main effect	Forage (whole plant)		
	Yield	N	S
	g pot^{-1}	------mg pot^{-1}------	
		Previous S fertilization	
No	13.05a*	258a	15.7a
Yes#	12.98a	254a	15.7a
		Current K fertilization	
Low (110)@	12.32b	252a	16.1a
High (220)	13.70a	261a	15.4a
		Current S fertilization	
Control (0)	5.42b	102c	6.2c
Gypsum (22.5)	14.40a	268b	16.2b
Gypsum (45)	15.14a	319a	20.1a
K_2SO_4 (22.5)	15.22a	286b	16.7b
K_2SO_4 (45)	14.88a	305a	19.4a

#Total amount of S applied in the field experiment in 1980, 1981, and 1983/84 was 200 kg ha^{-1}.
@Nutrient applied in the current fertilization, in mg kg^{-1} of soil.
*Means within columns in subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

in the first harvest, whereas the forage yield of the whole plant in the second harvest was increased by high K fertilization. This significant effect on forage yield was a consequence of significant increases in yields of stolons and roots (Table 5-4). Higher K application also significantly lowered N concentrations in the petioles + leaflets and stolons. This may be attributed to the dilution of N in the plant tissue. However, each plant part at the second harvest had a lower S concentration when higher K rate was

applied. Dilution effect may provide explanation for decreased S concentration in the stolons and roots, but certainly not for that in the petioles + leaflets. Also, decreases in the S concentration in any plant part exceeded that of N concentration, so that the N:S ratio was wider at the high than the low K fertilization.

Table 5-4. Effects of current K fertilization on yield, N and S concentrations, N:S ratio, and N and S contents in petioles + leaflets, stolons, and roots of white clover at the second harvest.

Current K fertilization	Forage					
	Yield	N	S	N:S	N	S
	g pot^{-1}	----g kg^{-1}----		ratio	--mg pot^{-1}--	
			(Petioles + leaflets)			
Low (110)@	5.24a*	21.0a	1.20a	17.7b	111a	6.5a
High (220)	5.44a	19.4b	1.05b	18.5a	106a	5.8b
			Stolons			
Low (110)	3.08b	13.6a	0.84a	17.2b	44b	2.5a
High (220)	3.95a	12.5b	0.63b	21.4a	51a	2.3a
			Roots			
Low (110)	4.01b	23.9a	1.74a	13.8b	96b	7.1a
High (220)	4.31a	23.8a	1.66b	14.4a	103a	7.2a

@Potassium applied in the current K fertilization, in mg kg^{-1} soil.
*Means within columns in subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

The most beneficial effects of any studied fertilization on white clover were observed at both harvests with the current S applications (Tables 5-2, 5-3, and 5-5). This agrees with the findings of Andrew (1977), Harris et al. (1968), and Tengku Maamun (1985). Plants grown in the

control pots had the lowest herbage yield, N and S concentrations, and N and S contents. In fact, they were only 27 and 36% of the maximum yield produced with the S application in the first and second harvests, respectively. The average numbers of petioles per pot in the control treatment were 124 and 141 at the first and second harvests, respectively, whereas the average when S was currently applied (there was no significant difference among them) 259 and 324, respectively.

Data obtained with petioles + leaflets at both harvests clearly showed the effect of the current S application (Tables 5-2 and 5-5). Both gypsum and potassium sulfate were highly effective. For most of the studied response variables the highest values were associated with the highest S rate (45 mg kg^{-1} of soil). Judged by the petioles + leaflets data, better performance of white clover was achieved at the first than at the second harvest. This can be attributed to the warmer days and/or to the lower K concentration in the plant tissue (to be discussed later) during the plant regrowth period.

Nitrogen concentrations in the petioles + leaflets from the control treatment were 18.7 and 18.6 g kg^{-1} plant tissue in the first and second harvests, respectively (Tables 5-2 and 5-5). These values are below normal for white clover, and were significantly increased by S application. Similar increases in N concentration as a consequence of S fertilization were observed with white clover stolons and roots

(Table 5-5). Andrew (1977), Shock et al. (1983), Spencer (1959), and Tengku Maamun (1985) attributed such increases to the improvement of the N_2-fixing system and/or N metabolism in the plant. Whether the benefit of S fertilization was direct in the nodulation and nodule functioning or indirect through the better plant development is not certain. The N_2-fixing system took advantage of the S

Table 5-5. Effects of current S fertilization on yield, N and S concentrations, N:S ratio, and N and S contents in petioles + leaflets, stolons, and roots of white clover at the second harvest.

Current S fertilization		Forage					
		Yield	N	S	N:S	N	S
		g pot^{-1}	----g kg^{-1}----		ratio	--mg pot^{-1}--	
				(Petioles + leaflets)			
Control	(0)@	2.62c*	18.6b	0.95c	19.5a	48c	2.5c
Gypsum	(22.5)	5.70b	19.4b	1.10b	17.9bc	111b	6.3b
Gypsum	(45)	6.32a	21.9a	1.26a	17.5c	137a	8.0a
K_2SO_4	(22.5)	5.90ab	19.3b	1.06b	18.3b	113b	6.3b
K_2SO_4	(45)	6.16a	21.7a	1.26a	17.5c	135a	7.7a
				Stolons			
Control	(0)	0.79b	10.2d	0.86a	12.8c	8c	0.6c
Gypsum	(22.5)	4.10a	12.8c	0.63b	21.2b	52b	2.5b
Gypsum	(45)	4.08a	14.8a	0.81a	19.4b	61a	3.2a
K_2SO_4	(22.5)	4.44a	13.5bc	0.59b	23.9a	59a	2.6b
K_2SO_4	(45)	4.17a	1.41ab	0.078a	18.9b	58ab	3.1a
				Roots			
Control	(0)	2.01b	22.8b	1.52c	15.0a	46a	3.0d
Gypsum	(22.5)	4.61a	23.1b	1.62b	14.2b	106b	7.4c
Gypsum	(45)	4.75a	25.4a	1.88a	13.5c	121a	8.9a
K_2SO_4	(22.5)	4.89a	23.2b	1.61b	14.4b	113ab	7.9bc
K_2SO_4	(45)	4.54a	2.48a	0.188a	13.2c	112ab	8.5ab

@Sulfur applied in the current fertilization, in mg kg^{-1}.
*Means within columns in subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

addition to the soil. By the second harvest, all roots grown in S fertilized pots had more and bigger nodules (Fig. 5-1).

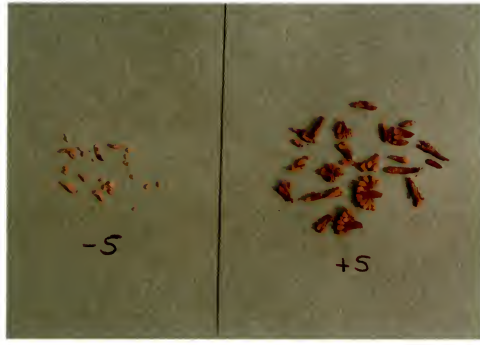

Fig. 5-1. Effect of current S fertilization on the nodule size of white clover (left= control, right= S added to the soil).

Sulfur concentration in the petioles + leaflets from the control treatment was far below the adequate level of this nutrient for white clover, based on the levels suggested by Andrew (1977), McNaught and During (1970), and Metson (1973). Although S application resulted in significant increases in its concentration, adequate S concentrations occurred only at the first harvest. The lower S concentration at the second harvest may have been related to the lower K in the plant tissue (to be discussed later). This agreed with the results from the previous greenhouse experiment (Chapter IV), which showed that when K concentra-

tion in the plant tissue was low even the highest S rate failed to bring S concentration in the plant top to an adequate level. Gallaher and Arias (1985) reported that S deficiency in maize (Zea mays L.) resulted in exclusion of K in leaf tissue. From the results obtained in the present experiments (Chapters IV and V), it seems quite clear that low K concentration in the petioles + leaflets prevents adequate S accretion even if the S fertilizer is sufficient.

Significant increases in the N and S contents in the white clover were observed as a consequence of S fertilization. The improvement was particularly large when either gypsum or potassium sulfate was supplied at 45 mg S kg^{-1} of soil.

The N:S ratio in the petioles + leaflets decreased in both harvests as the S rates increased. Most of the researchers have considered a N:S range of 16:1 to 19:1 in these tissues as an indication of adequate S nutrition. It is shown in Tables 5-2 and 5-5 that the ratios from all S treatments fell in this range while those from the control were higher. However, data obtained at the second harvest suggested that besides N:S ratio, the S concentration should also be examined for diagnostic purposes.

The yields, N concentrations, and N contents in stolons and roots were significantly higher from the S fertilization than from the control (Table 5-5). The same was true for S concentration and content in roots. Among the three different tissues from the second harvest, roots had the highest N

and S concentrations, and stolons the lowest. There was no significant difference in S concentration in the stolons between the control and the high rate of S fertilization. The N:S ratio in the stolons was very low in the control and relatively high in the S treatment. However, high rate of S reduced the N:S ratios.

At the first harvest, correlations between N and S concentrations as well as N and S contents in the petioles + leaflets were highly significant (0.97*** and 0.99***, respectively). At the second harvest the coefficients for the correlation between N and S concentrations in the petioles + leaflets, stolons, and roots, were 0.88***, 0.26, and 0.78***, respectively. Those between N and S contents were 0.97***, 0.83***, and 0.97***, respectively. When the contents of N and S in the whole plant at the second harvest were calculated, the correlation coefficient was 0.96***. Once more, these values confirmed that more N was fixed and/or metabolized in the legume when more S was taken up by the plant.

It has been reported (Jones, 1962) that sulfate accumulates in the stolons of clover when S is supplied at high rates. In the present experiment, stolons had a very low S concentration. Its N:S ratio was not a good diagnostic measure of S deficiency. Therefore, total S concentrations and N:S ratios of petioles + leaflets may be more adequate for diagnostic purposes.

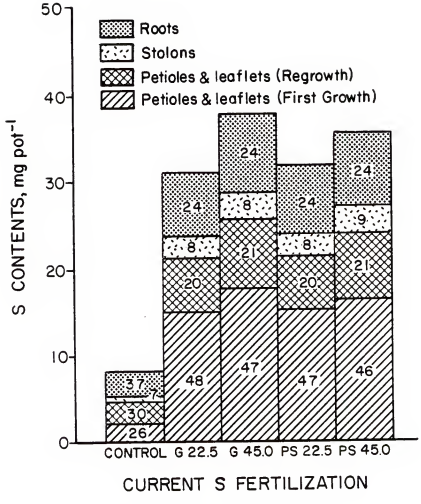

Fig. 5-2. Sulfur distribution in various tissues of white clover. Numbers within the bars indicate percentage contribution. (G= gypsum, PS= potassium sulfate; 22.5 and 45.0 are S rates in mg kg^{-1}).

The distribution of S in plant parts is shown in Fig. 5-2. Total S content in the plants without S application was only 22 to 26% of those with S. Although the S content varied among the S treatments the percentage of S in each plant part was nearly constant. In fact, 46 to 48% of the total S content in the plant was in the petioles + leaflets at the first harvest, 20 to 21% in the petioles + leaflets at the second harvest, 8 to 9% in the stolons, and 24 to 25% in the roots. Only the control treatment had a somewhat different distribution, with the respective plant parts containing 26, 30, 7, and 37% of the total plant S content.

In the previous greenhouse experiment (Chapter IV), white clover grown in soil without S fertilization did not bloom at the harvest time. Robinson and Jones (1972) reported retardation of flowering time in S-deficient Stylosanthes humilis H. B. K.. In the present experiment the number of flower heads was significantly different between S-treated plants and those without S (Fig. 5-3). By the time of the first harvest, out of 16 pots not receiving current S fertilization, plants from only two pots had a single flower heads. During the regrowth period, 11 had plants with one or more flower heads. In the no-S treatment, flowering was not only delayed, but also the number of flower heads was drastically reduced. Considering that white clover needs to produce enough seeds to generate new plants

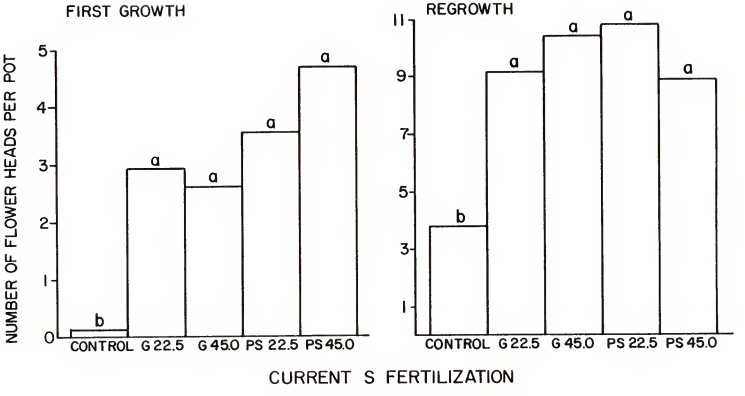

Fig. 5-3. Number of flower heads per pot during first growth and regrowth of white clover (G= gypsum, PS= potassium sulfate; 22.5 and 45.0 are S rates in mg kg-1 of soil).

during the following winter, S fertilization can be essential to the persistence of white clover in the sward.

In addition to the yield difference between those white clover plants with and without current S treatment, visual symptoms of S deficiency were noticeable. After the initial 5 weeks of growth, plants in the control pots were easily distinguishable from those of S treatments. Sulfur-deficient plants were smaller, and had yellowish leaflets and reddened petioles and stolons. Reduced tillering, delayed flowering, reduced number of flower heads, and less numerous and smaller nodules were also characteristic of S-deficient plants.

Forage K concentrations and contents in the petioles + leaflets collected at both harvests are presented in Tables 5-6 and 5-7. The interactions between previous S fertilization and current S fertilization, and between current K fertilization and current S fertilization were highly significant. Potassium concentration and content in the plant material were much higher at the first than at the second harvest. Plants grown on currently S-treated soils had K concentration at the second harvest that can be considered inadequate for white clover (Martin and Matocha, 1973). Plants in the control treatment grew poorly, and showed K accumulation at both harvests. When rates of current S treatment were equal, plants developed on previously S-treated soils had lower K concentration and content than those on soils that did not receive S previously

5-6). Similarly, white clover fertilized with higher K rate showed higher K concentration and content than plants with lower K rate (Table 5-7). However, even the high K rate (220 mg kg^{-1} of soil) did not result in adequate K concentration of white clover at the time of the second harvest.

Table 5-6. Effects of current S fertilization on forage K concentrations and contents in petioles + leaflets of white clover at the first and second harvests.

Current S fertilization	Forage K			
	First harvest		Second harvest	
	g kg^{-1}	mg pot^{-1}	g kg^{-1}	mg pot^{-1}
	Previous S fertilization = No			
Control (0)@	40.2a*	91c	26.4a	68a
Gypsum (22.5)	25.3b	208a	5.2b	29b
Gypsum (45)	22.2c	201ab	4.2b	28b
K_2SO_4 (22.5)	22.9c	194ab	4.4b	26b
K_2SO_4 (45)	22.2c	192b	4.4b	28b
	Previous S fertilization = Yes#			
Control (0)	39.8a	99c	20.0a	53a
Gypsum (22.5)	19.2b	168b	4.2bc	24b
Gypsum (45)	17.1b	142b	3.6bc	22b
K_2SO_4 (22.5)	18.4b	153b	4.5b	27b
K_2SO_4 (45)	17.2b	151b	3.3c	20b

@Sulfur applied in current fertilization, in mg kg^{-1} of soil.
#Total amount of S applied in the field experiment in 1980, 1981, and 1983/84 was 200 kg ha^{-1}.
*Means within columns in subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Table 5-7. Effects of current S fertilization at each rate of current K fertilization on forage K concentrations and contents in petioles + leaflets of white clover at the first and second harvests.

Current S fertilization	Forage K			
	First harvest		Second harvest	
	g kg^{-1}	mg pot^{-1}	g kg^{-1}	mg pot^{-1}
	Current K fertilization = Low (110)@			
Control (0)@	39.1a*	93b	17.9a	44a
Gypsum (22.5)	14.2b	118a	3.4b	20b
Gypsum (45)	14.5b	125a	3.3b	20b
K_2SO_4 (22.5)	14.1b	118a	3.3b	20b
K_2SO_4 (45)	14.7b	124a	3.3b	20b
	Current K fertilization = High (220)			
Control (0)	40.9a	97c	28.4a	77a
Gypsum (22.5)	28.2b	231ab	6.0b	34b
Gypsum (45)	27.0bc	244a	4.5b	30b
K_2SO_4 (22.5)	27.2bc	229ab	5.6b	33b
K_2SO_4 (45)	24.7c	219b	4.5b	28b

@Nutrient applied in the current fertilization, in mg kg^{-1} of soil.
*Means within columns in subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Calcium, Mg, and P concentrations of white clover at the two harvests are presented in Table 5-8. Concentrations of these three nutrients in all plants, regardless of treatments,occurred in a range considered adequate for white clover (Martin and Matocha, 1973). The increase in rate of current K fertilization resulted in consistent and significant decreases of Ca and Mg concentrations. Plant P concentration was significantly higher in the control treatment than in treatments where gypsum or potassium sulfate was applied. At the first harvest, more Ca accumulated in the

poorly-grown plants from the control treatment than in plants where S was applied, but at the second harvest both Ca and Mg were present at lower concentrations. It is noteworthy that K concentration in the legume had shown opposite behaviour.

Table 5-8. Main effects of previous S fertilization, and current K and S fertilizations on Ca, Mg, and P concentrations in petioles + leaflets of white clover at the first and second harvests.

Main effect	Forage (petioles + leaflets)					
	First harvest			Second harvest		
	Ca	Mg	P	Ca	Mg	P
	----------g kg^{-1}----------					
			Previous S fertilization			
No	18.3a*	4.6a	4.1a	25.6a	7.6b	4.8a
Yes#	18.2a	4.8a	3.5b	25.9a	8.0a	4.9a
			Current K fertilization			
Low (110)@	19.2a	5.0a	3.9a	27.1a	8.5a	5.1a
High (220)	17.3b	4.4b	3.7a	24.5b	7.1b	4.7a
			Current S fertilization			
Control (0)@	21.0a	4.9a	5.2a	21.1c	5.9b	8.5a
Gypsum (22.5)	18.2b	4.7a	3.4b	27.9a	8.5a	4.3b
Gypsum (45)	17.9b	4.6a	3.5b	27.5a	8.2a	3.8b
K_2SO_4 (22.5)	16.8c	4.7a	3.4b	25.6b	8.2a	4.0b
K_2SO_4 (45)	17.2bc	4.7a	3.4b	26.7ab	8.2a	3.7b

#Total amount of S applied in the field experiment in 1980, 1981, and 1983/84 was 200 kg ha^{-1}.
@Nutrient applied in the current fertilization, in mg kg^{-1} of soil.
*Means within columns in subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Table 5-9. Effects of current S fertilization at each or previous S fertilization, and at each rate of current K fertilization on soil Mehlich-I extractable K.

Current S fertilization	Soil Mehlich-I extractable K			
	Previous S fertilization		Current K fertilization	
	No	Yes#	Low(110)@	High(220)
	----------cmol (p^+) kg^{-1}----------			
Control (0)@	0.16a*	0.17a	0.10a	0.24a
Gypsum (22.5)	0.10b	0.16ab	0.09a	0.16b
Gypsum (45)	0.10b	0.16ab	0.09a	0.17b
K_2SO_4 (22.5)	0.11b	0.15b	0.10a	0.16b
K_2SO_4 (45)	0.14ab	0.16ab	0.12a	0.18b

#Total amount of S applied in the field experiment in 1980, 1981, and 1983/84 was 200 kg ha^{-1}.
@Nutrient applied in the current fertilization, in mg kg^{-1} of soil.
*Means within columns in subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Mehlich-I extractable soil K at the end of the experiment in each S treatment related to both previous S and current K fertilization is shown in Table 5-9. Both soils that received S previously and high K currently had higher extractable K than those without previous S previously and with low K. The lower K values in the soil without previous S fertilization can be attributed to higher K uptake by the plants growing on this soil, especially at the first harvest (Table 5-6). On the other hand, the current high K fertilization provided much more K to the soil than the low K rate (440 vs 220 mg K pot^{-1}), so that both K uptake by the plants and extractable soil K at the end of the experiment were higher. Where gypsum was used as the source, K was supplied

as KCl. The same K source was used to supplement K_2SO_4 to obtain the desired K rate. Based on soil test interpretation by Rhue and Kidder (1983), the extractable K obtained under the current high K application can be rated as medium for most crops. It is surprising that white clover had an inadequate K concentration. However, Williams and Steinbergs (1959) reported no yield response of oats to S, when the extractable K in the soil was 0.19 cmol (p^+) kg^{-1} of soil.

Soil pH, organic C, total N, total and phosphate-extractable S, and Mehlich-I extractable Ca, Mg, and P are shown in Table 5-10. Current K fertilization did not affect significantly any of these soil parameters. All values were significantly higher from the soil that had received S previously. When compared with the treatment without currently applied S gypsum or potassium sulfate significantly decreased soil pH both in water and in KCl, and Mehlich-I extractable Ca, Mg, and P. Soil pH in water progressively decreased as the gypsum rate was increased; similar results were obtained in the greenhouse experiment described in Chapter IV. Decreases of extractable Ca, Mg, and P with currently supplied S can be attributed to higher uptake of these nutrients by the legume. Both total and phosphate-extractable soil S were significantly increased with the increase in rate of currently applied S. The highest S fertilization rate resulted in extractable S levels of 20.4 to 21.4 mg kg^{-1} of soil at the end of the experiment. These levels are far above the critical value of 10 to 12 mg kg^{-1}

Table 5-10. Soil pH (2:1 in water or 1 M KCl), organic C, total N, total and phosphate-extractable S, and Mehlich-I extractable P, Ca, and Mg at the end of the experiment.

Main effects	pH		O.C.	Total N	S		Mehlich-I Ext.Φ		
	H_2O	KCl			Tot.	Ext.	P	Ca	Mg
			g kg^{-1}		mg kg^{-1}			cmol(p^+)kg^{-1}	
Previous S fertilization									
No	5.4b*	5.0b	20.5b	1073b	150b	9.7b	30b	5.15b	0.62b
Yes#	5.5a	5.1a	21.7a	1202a	162a	12.7a	37a	5.86a	0.72a
Current K fertilization									
Low (110)@	5.5a	5.1a	21.2a	1158a	154a	11.5a	34a	5.55a	0.66a
High (220)	5.5a	5.0a	21.0a	1116a	157a	11.9a	34a	5.47a	0.68a
Current S fertilization									
Control (0)	5.7a	5.2a	21.3a	1147a	146c	2.1c	41a	5.91a	0.87a
Gypsum (22.5)	5.4b	5.0b	20.9a	1127a	155b	5.3b	32b	5.52b	0.62b
Gypsum (45)	5.3c	5.0b	21.2a	1115a	164a	20.4a	32b	5.48b	0.61b
K_2SO_4 (22.5)	5.4b	5.0b	21.5a	1170a	153b	6.7b	32b	5.38b	0.61b
K_2SO_4 (45)	5.4b	5.0b	20.6a	1128a	165a	21.4a	32b	5.26b	0.61b

ΦStands for Extractable.
#Total amount of S applied in the field experiment in 1980, 1981, and 1983/84 was 200 kg ha^{-1}.
@Nutrient applied in the current fertilization, in mg kg^{-1} soil.
*Means within columns in subtables followed by the same letters are not significantly different at the 5% level of probability (DMRT).

of soil for temperate legumes as suggested by Fox et al. (1964), Grava (1971), and Walker and Doornenbal (1972). Therefore, adequate available S should have been present for white clover even at the end of the experiment. Correlations of extractable soil S with S content in petioles + leaflets at both the first and second harvests and with S content in the whole plant at the second harvest showed values of 0.56***, 0.58***, and 0.61***, respectively.

Organic C and total N in the soil did not change significantly due to current S treatments, but their values were relatively smaller than those observed in the soil analyzed before the experiment started, suggesting that net mineralization of organic matter occurred during the experiment.

The apparent S recoveries from fertilizer by white clover in two harvests were 51 and 33% when gypsum was supplied at 22.5 and 45 mg S kg^{-1} of soil, respectively. When potassium sulfate was applied, the values were 52 and 30%, respectively. In the soil, the respective S recoveries were 20, 20, 16 and 21%. This means that 29 to 32% of the S supplied by the fertilizer when the S rate was 22.5 mg kg^{-1} of soil, and 47 to 49% when the rate was 45 mg S kg^{-1} of soil could not be accounted for through plant uptake and soil total soil S. It is noteworthy to point out that no leaching loss was allowed from the experimental pots.

Summary

A greenhouse experiment was carried out with white clover grown on a Spodosol with and without S applied for 4 years before the experiment. Rates of 110 and 220 mg K kg^{-1} of soil, and rates of 0, 22.5 and 45 mg S kg^{-1} of soil constituted current fertilization. Gypsum and K_2SO_4 were used as S sources. Potassium was supplied as K_2SO_4 and/or KCl. Previous S fertilization had no significant effect either on the yield or N and S contents in petioles + leaflets at the first harvest and those of the whole plant at the second harvest. Stolon and root yields, K concentration, and K content were higher when plants were grown with high K fertilization but Ca and Mg concentrations were lower. Current S fertilization resulted in the most beneficial effects for the legume. Forage yield, N and S concentrations, and N and S contents were increased by S application. Nitrogen and S contents were the highest when S rate was 45 mg kg^{-1} of soil. Visual symptoms of S deficiency were evident in the leaflets, petioles, and stolons of white clover in the non-S-treated pots. Plants also showed reduced tillering, delayed flowering, reduced number of flower heads, and reduced number and size of the nodules when S was not applied. Sulfur concentrations in the petioles + leaflets were adequate at the first harvest when K nutrition was adequate, but S did not reach adequacy at the second harvest when a lowering of K concentration occurred.

The N:S ratios of the petioles + leaflets from currently S-fertilized soil ranged between 16.3:1 and 18.3:1, whereas the ratio exceeded 19:1 in soil that did not receive S fertilization.

CHAPTER VI
CHANGES AND DISTRIBUTION OF EXTRACTABLE NUTRIENTS, pH, AND ORGANIC CARBON IN A SPODOSOL PROFILE UNDER A LONG-TERM DIFFERENTIALLY LIMED AND FERTILIZED LEGUME-GRASS SWARD.

Introduction

Florida Spodosols support native species such as pine trees (Pinus spp.), saw-palmetto (Serenoa repens Bartr. Small), runner oak (Quercus minima Sarg.), gallberry (Ilex glabra L.), and have been modified for the production of vegetables, annual crops, citrus, and improved pastures. Pensacola bahiagrass (Paspalum notatum Flugge) alone or associated with white clover (Trifolium repens L.) has usually been the choice for pastures on the Spodosols in North Central Florida. One advantage of including legumes in pastures is that they may supply substantial amounts of N to the soil and to the companion grass through the symbiotic N_2-fixation.

For the establishment of permanent pastures, liming materials are usually broadcast and incorporated into the top layer of the soil while fertilizers are mainly broadcast on the soil surface without incorporation. Subsequently, both lime and fertilizers are periodically surface applied to the pastures.

Soil fertility improvements have been reported for periodically limed and fertilized permanent pastures. Koger et al. (1970) in Florida showed significant increases in organic matter and N in the top 15 cm of soil after 6 years of white clover-Pensacola bahiagrass pasture management (including liming and fertilization). Bryan and Evans (1971) in Australia found increases in organic matter, N, P, K, Ca, and S in the 0 to 10 cm soil depth when they compared fertilized grazed pastures with the virgin area, after a 11- or 15-year period.

Nutrient distribution in a soil profile is affected by soil characteristics such as cation and anion exchange capacity and rate of water percolation, by lime and fertilizer rates, sources, and frequency of application, by the specific nutrient mobility, by rainfall amount, and by the kind of vegetation on the soil (Barrow, 1980; Chao et al., 1962; Coleman et al., 1958; Tisdale et al., 1985).

Koger et al. (1970) sampled seven layers within the top 53 cm of the profile of a Myakka fine sand used for 9 years as grazed pasture. They found that organic matter and ammonium acetate-extractable Ca, Mg, and K accumulated in the first four layers within 15 cm of the soil surface, but little measurable effect of liming and fertilization occurred for the soil sampled below the 15 cm depth. Ozanne (1980) pointed out that the bulk of P topdressed on soils used for forage production is usually retained in the surface 2 to 3 cm of soil.

Fox et al. (1964) sampled a profile of a Latosol (Oxisol) at 15-cm intervals to 120 cm and observed that phosphate-extractable S occurred in substantial concentration throughout the sampled layers.

Studies on Cu, Fe, Mn, and Zn distribution in the soil profile are scarcer than for the macronutrients. Micronutrients are known to be bound to organic substances in the soil, and the complexes formed can alter micronutrient movement in the soil profile (Stevenson and Ardakani, 1972).

The principal objective of this experiment was to study the changes and distribution of extractable nutrients, pH, and organic carbon in a Myakka fine sand profile that has supported a permanent sward for 32 years, under differential liming and fertilizer treatments.

Materials and Methods

The experimental area was established in 1952, and contained white clover and Pensacola bahiagrass. It has been designated as Sward I, and has been described in Chapter III, experiment no. 1.

Soil samples were taken in May 1984, at nine soil depths: 0 to 2.5, 2.5 to 5.0, 5.0 to 7.5, 7.5 to 15.0, 15.0 to 30.0, 30.0 to 45.0, 45.0 to 60.0, 60.0 to 75.0, and 75.0 to 90.0 cm. Four main plots, each having two subplots, and replicated four times, were sampled at each soil depth.

The four main plots were characterized as follows:

1. No lime and no micronutrients were applied. White clover did not establish, and Pensacola bahiagrass was the only vegetation. This treatment corresponded to treatment no. 1 in the original experiment, and is designated hereafter as the control (unlimed) treatment;

2. This treatment received lime in 1952 and was limed periodically after 1952 to maintain soil pH near 6.0. The total amount of liming material applied through the years was aproximately 15 Mg ha^{-1}. Micronutrient-containing fertilizers were not applied. Both white clover and Pensacola bahiagrass grew well. It corresponded to the original treatment no. 2, and it is designated as lime treatment in this study.

3. This treatment received lime, and had a mixed sward of white clover and bahiagrass as in no. 2 above. A mixture of Cu, Fe, Mn, and Zn sulfates was applied in 1952 to supply 4.2, 3.2, 4.6, and 3.9 kg ha^{-1} of each micronutrient, respectively. Copper and Zn were reapplied in 1955, 1958, and 1959, through the same sources and at the same rates. The total application of Cu, Fe, Mn, and Zn corresponded to 16.8, 3.2, 4.6, and 15.6 kg ha^{-1}, respectively. In 1973, B, Cu, Fe, Mn, and Zn were inadvertently distributed in the area at 0.3, 0.3, 1.6, 0.7, and 0.6 kg ha^{-1}, respectively. This treatment was treatment no. 4 in the original experiment, and it is hereafter called lime + MN.

4. This treatment received lime, Cu, Fe, Mn, and Zn, and supported a mixed sward as described in no. 3 above. Borax was applied in 1952, 1955, 1958, and 1959 to supply 1.2 kg B ha^{-1} each time or a total of 4.8 kg B ha^{-1}. Sulfur was added at 30 kg ha^{-1} as gypsum in December 1982. It corresponded to treatment no. 6 in the original experiment, and is designated as lime + MNS in the present study.

Subplots were with and without S fertilizer during the last 4 years. Where S was applied, rates were 40 kg ha^{-1} in November 1980, 30 kg ha^{-1} in March 1981, 40 kg ha^{-1} in November 1981, 30 kg ha^{-1} in November 1983, 30 kg ha^{-1} in January 1984, and 30 kg ha^{-1} in March 1984. In the November 1980, 1981, and 1983 fertilizations, S was supplied as gypsum, whereas potassium sulfate was used in the other applications.

Phosphate and K fertilizers were applied at establishment of the experiment in 1952, and were reapplied annually to all experimental plots. The total P and K applied over the years amounted to 1530 and 5950 kg ha^{-1}, respectively.

Soil samples were air-dried, crushed when necessary, passed through a 2-mm sieve, and kept in paper bags. Twenty grams of soil were used to determine pH in a suspension with 40 mL of water. After that, 2.98 g of analytical grade-KCl was added to the suspension, which was stirred and left standing for 1 hour before reading pH again. For Ca, Mg, K, P, Cu, Fe, Mn, and Zn determinations, soil samples were extracted with Mehlich-I double-acid (0.05 M HCl + 0.025 M

H_2SO_4) extractant (Nelson et al., 1953). Sulfate-S was extracted with a 500 mg P L^{-1} as $Ca(H_2PO_4)_2.2H_2O$ solution (Fox et al., 1964), and determined by a turbidimetric method (Butters and Chenery, 1959; Massuomi and Cornfield, 1963).

Soil samples were ground to pass a 0.5-mm screen before organic C, total N, and total S were determined. Organic C was determined by the Walkley-Black procedure (Walkley, 1947), total N was determined by the semimicro- Kjeldahl digestion method (Nelson and Sommers, 1972), and total S by the direct combustion method in a Leco SC-132 Sulfur Analyzer (Leco Corporation, 1980).

Analysis of variance and mean comparisons were done according to statements given by Freund and Littell (1981) for a split-plot experimental design. The lime-MN-S treatments constituted the main plots while the S application and depth of soil sampling were the subplots.

Results and Discussion

Significance of the F tests for the main effects of lime and micronutrients treatments, sulfur fertilization, and depth of soil sampling and for their interactions on the response variables studied are presented in Table 6-1. Data on these variables are presented in Tables A-1 to A-8 in the Appendix.

Soil organic carbon, as well as C:N and C:S ratios changed significantly only with soil depth (Fig. 6-1). Significant decreases in organic carbon were obtained with soil

depth to the 30 to 45 cm layer with no further changes down to 90 cm. Blue et al. (1964) sampled a Myakka fine sand down to 60 cm, 10 years after white clover-Pensacola bahiagrass establishment. They observed no significant changes in the organic C as the soil depth was increased further than 15 cm. It seemed possible that more organic C would have reached deeper soil layers as the time progressed.

Table 6-1. Significance of the F tests obtained in the analysis of variance for the main effects and interactions among lime-MN-S treatments (T), sulfur application (S), and soil depth (D).

Response variable	T	S	D	TxS	TxD	SxD	TxSxD
pH in H_2O	**[a]	ns[b]	**	ns	**	ns	ns
pH in KCl	**	ns	**	ns	**	ns	ns
Extractable Ca	**	ns	**	ns	**	ns	ns
Extractable Mg	**	ns	**	ns	**	ns	ns
Extractable K	**	**	**	**	**	**	ns
Extractable P	**	ns	**	ns	**	ns	ns
Extractable Cu	**	ns	**	*	**	ns	ns
Extractable Fe	ns	*[a]	**	ns	**	ns	ns
Extractable Mn	**	*	**	ns	**	**	ns
Extractable Zn	**	*	**	ns	**	ns	ns
Extractable S	ns	**	**	ns	ns	**	ns
Organic C	ns	ns	**	ns	ns	ns	ns
Total N	**	ns	**	ns	**	ns	ns
Total S	ns	ns	**	ns	**	ns	ns
C:N ratio	ns	ns	**	ns	ns	ns	ns
C:S ratio	ns	ns	**	ns	ns	ns	ns
N:S ratio	**	ns	**	ns	*	ns	ns

a Statistically significant at 5 and 1% level of probability.
b Statistically not significant at the level of probability less or equal to 5%.

Blue et al. (1964) and Blue (1979) found significant increases in organic C in the top 15 cm of soil after 10 and 25 years of undisturbed white clover-Pensacola bahiagrass sward. They considered plant roots and crowns as the primary source for the organic C accumulation over the years. In the present experiment, organic C was much higher when determined closer to the surface soil. The same trend was reported by Koger et al. (1970). Pensacola bahiagrass has thick stolons close to the soil surface, and usually loses leaves on the surface. Once dead and decomposed, these plant materials can make substantial contributions to organic C in the top soil layers.

Both the C:N and C:S ratios changed less than the organic C concentration in the soil layers, and did not show any significant difference within the top 15 cm of soil. However, they increased as the soil depth increased to the 60 to 75 cm layer. This increase in the C:N ratio with soil depth is divergent to findings in most soils (Stevenson, 1982), but was also reported by Hinman (1964) in five Canadian soils.

Determination of C:S ratio in soils have been confined mostly to the top soil layer, and has been shown to vary between 10:1 and 200:1 among soils (Jones et al., 1975, Whitehead, 1964). In the present study, values for this ratio ranged between 139 and 391 throughout the sampled profile. The highest values occurred between 15 and 75 cm soil depth.

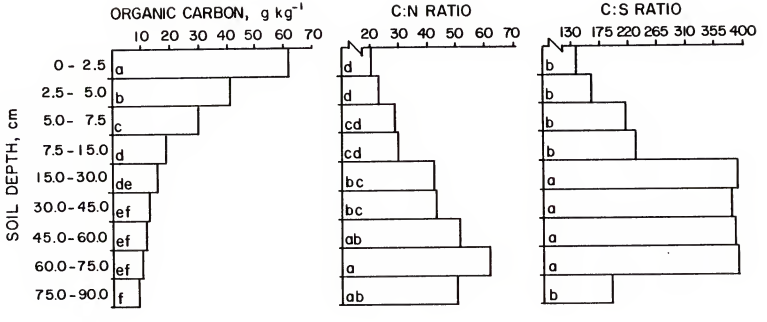

Fig. 6-1. Distribution of organic C, and C:N and C:S ratios in the Myakka fine sand profile. Data represented by bars within figures with the same letters are not significantly different at the 5% level of probability (DMRT).

Neither organic carbon concentration nor its relationship with N or S was significantly changed by lime-micronutrient-S combinations or sulfur fertilization. Among the lime-MN-S combinations, the control plots had much lower soil pH, had no white clover in the sward, and had much lower annual forage yields than any limed plot. Sulfur fertilization resulted in higher forage yield in the mixed sward (Chapter III). However, those increases in plant production as a consequence of lime and sulfur applications did not correspond to increases in soil organic C.

Total soil N, and S as well as their ratios were affected by lime-MN-S combinations and soil depth (Fig.6-2). Total N was higher in limed soils (lime or lime + MNS) than in the control at the 0 to 2.5, 2.5 to 5.0, and 5.0 to 7.5 cm soil depths. Plots under lime and lime + MNS treatments contained white clover in the sward, and the increase in total N in the soil is likely due to symbiotic N_2-fixation. Blue (1979) reported significant increases in total soil N in the limed plots in the same experimental area over the 25-year period, and estimated that the N fixed by the white clover approximated 275 kg ha^{-1} $year^{-1}$. Total N decreased with increasing soil depth until the 15 to 30 cm soil layer, and no further significant change was observed down to 75 to 90 cm. Stevenson (1982) suggested that the protein content of the soil organic matter may be higher in the surface soil than in the subsoil of Spodosols. Hinman (1964) also

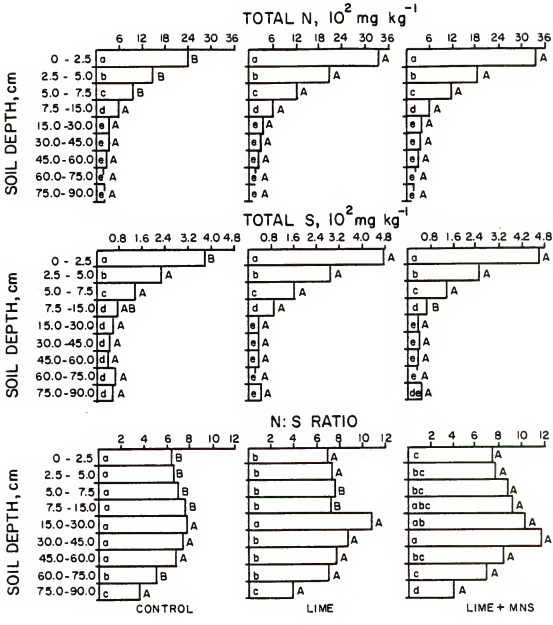

Fig. 6-2. Effects of lime-MN-S combinations and soil depth on total N, total S, and N:S ratio in the Myakka fine sand profile. Bars with the same lower-case letters within subfigures, and bars followed by the same upper-case letters within soil depth are indicative of no significant difference among means at the 5% level of probability (DMRT).

reported progressive decreases in total N in five Canadian soils, as the soil depth increased to 120 cm.

Total soil S was higher in limed soils than in the unlimed soils, at the 0 to 2.5 cm depth. In the limed soils, total S decreased with increasing soil depth to 15 to 30 cm. Significant decreases in the total S were observed from the surface soil down to the 7.5 to 15 cm layer in the unlimed plots. Much of the total S in soils of the humid regions of the world occurs in organic forms (Stevenson, 1982), and just like organic C and total N, it was found in higher concentrations in the top layers of the soil profile.

Total S in the soil did not vary significantly with the application of 200 kg S ha^{-1} to the sward from 1980 to 1984. Part of the applied S (about 13%) was removed with the harvested forages (Chapter III, experiment no. 1). The average total S over all the lime-Mn-S combinations and soil depths was 126 mg kg^{-1} in the subplots under no S applications, and 122 mg kg^{-1} in the S-fertilized subplots. This suggested that a major portion of the applied S was leached out of the 0 to 90 cm soil depth.

The soil N:S ratio was significantly higher in the limed (lime and lime + MNS) than in control plots at the 0 to 2.5 and 2.5 to 5.0 cm soil layers. In the 5.0 to 7.5 and 7.5 to 15.0 cm layers, the lime + MNS treatment had higher N:S ratio than the others. Since total N was significantly higher in the top layers of the limed soils than in the unlimed plots, and the changes in total S did

not follow the same pattern in the same soil layers, such differences in the N:S ratio between limed and unlimed plots were not surprising. Within each of the lime-MN-S combination, except for the 75 to 90 cm soil layer, the N:S ratio was in most cases hardly differentiated among soil layers. In the limed soils, the N:S ratio was significantly higher or tended to be higher in the 15 to 30 cm and 30 to 45 cm soil layers.

Phosphate-extractable S was significantly affected by S fertilization and soil depth interaction. The soil layers above the 15 cm soil depth, and at the 75 to 90 cm soil layer, subplots fertilized with S had higher extractable S than those not fertilized (Fig. 6-3). Although the differences between S-treated and non-S-treated subplots had shown statistical significances in some soil layers, it is worthy to note that extractable S in all soil layers, except that at 75 to 90 cm, is considered very low for forage growth (Grava, 1971; Martin and Matocha, 1973). Some contribution of S to forages with deep root system, such as Pensacola bahiagrass, can be expected at the 75 to 90 cm soil layer, especially under S fertilization. In fact, residual effect of the previous S applications was observed in the yield of the mixed sward in 1983 (Chapter IV, field experiment). During soil sampling, it was observed that the 75 to 90 cm depth always had higher soil moisture, and even was wet in two eastern replications of the experiment. This soil layer seemed to have higher content of fine soil particles, than

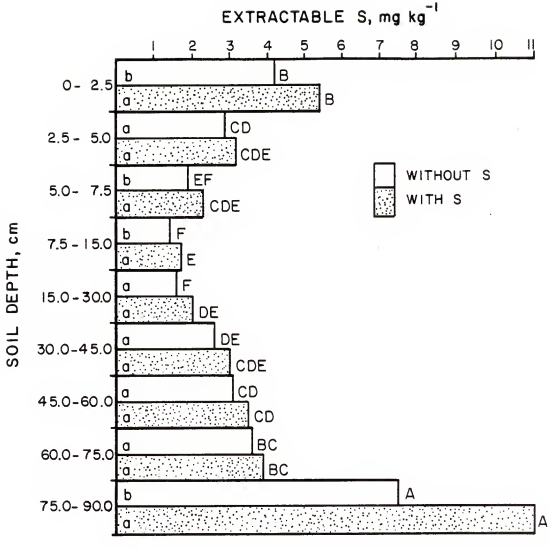

Fig. 6-3. Effects of sulfur fertilization and soil depth on extractable S in the Myakka fine sand. Bars with the same lower-case letters within soil depth, and bars followed by the same upper-case letters within S treatments are indicative of no significant difference among means at the 5% level of probability (DMRT).

the top soil layers. Also, the pH in water of that soil ranged between 5.0 and 5.1. Probably, part of the leached S from the top layers was adsorbed at the 75 to 90 cm soil layer. Conversely, the soil layers around the 15 cm soil depth always showed the lowest extractable S.

Jordan and Bardsley (1958) sampled Red-Yellow Podzolic soils (Ultisols) at 15-cm intervals down to 90 cm, from 29 experimental areas in the southeastern United States. They observed very low extractable S in the top 15 cm of the soil, and S accumulation in deeper layers of the soil (probably related to the occurrence of the Bt horizon). Bardsley and Kilmer (1963) sampled 21 profiles at depth as low as 60 cm in the same region. They had a Ground-water Podzol (Spodosol) among the soils, and reported low extractable S in the surface of such soil with appreciable increases at the 30 to 45 cm and 45 to 60 cm layers. These authors observed that increases in soil acidity and clay content were associated with higher extractable S at deeper layers in most of the soils studied. Also, Kamprath et al. (1956) found that S adsorption is inversely related to soil pH and P level in the soil. In fact, in the 75 to 90 cm soil depth, soil pH was about 5.0-5.1 and extractable P ranged from 6 to 21 mg kg^{-1} of soil.

Soil pH (in water and in 1 M KCl) significantly changed with lime-MN-S treatments and soil depth interaction (Fig. 6-4). Soil pH in water exceeded pH in KCl by 0.7 to

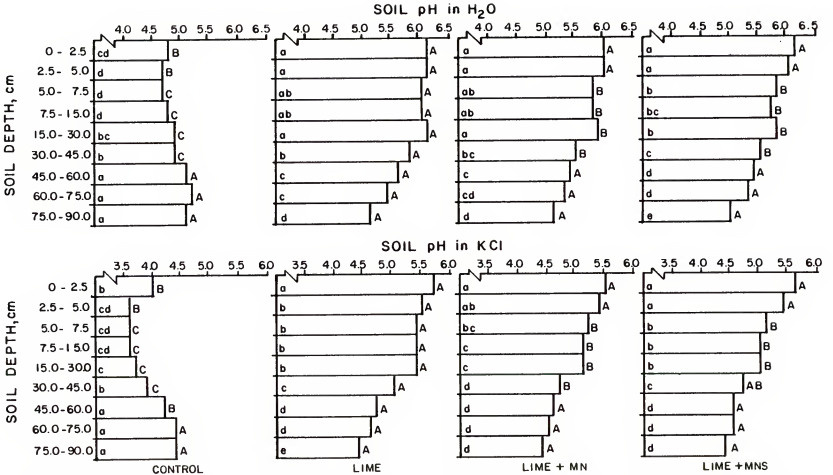

Fig. 6-4. Effects of lime-MN-S combinations and soil depth on soil pH in water and in 1 M KCl in the Myakka fine sand profile. Bars with the same lower-case letters within subfigures, and bars followed by the same upper-case letters within soil depth are indicative of no significant difference among means at the 5% level of probability (DMRT).

1.2 units in the unlimed plots, and by 0.4 to 0.9 units in the limed soils. Except for the 45 to 60 cm, 60 to 75 cm and 75 to 90 cm soil depth, the limed soils had higher pH values than the unlimed, at all soil depths. The surface-applied lime over the years moved into the profile, and increased soil pH down to 45 cm. In the spodic horizon (occurring right below the 30 to 45-cm soil layer), no significant effect of the lime was observed. In the unlimed plots, the highest pH values were observed at the three deeper soil layers (between 45 and 90 cm). Yuan (1963) found that virgin Leon fine sand (now Myakka fine sand) had higher pH in water and in KCl at 65 to 80 cm or 65 to 105 cm depths than at 0 to 5 cm or 0 to 10 cm, respectively. However, limed soils had higher pH in the layers close to the surface. Also, soil pH decreased with increasing soil depth in the limed plots.

Mehlich-I extractable macronutrients and micronutrients in the soil were significantly affected by lime-MN-S treatments and soil depth interactions. Limed soils had higher extractable Ca and Mg than unlimed soils in each soil layer from the surface to 45 cm (Fig. 6-5). A similar pattern was observed for soil pH. Extractable Ca decreased with increasing soil depth to 30 cm in unlimed plots, and to 45 cm in limed plots. Below those depths, extractable soil Ca was very low irrespective of liming or S applications. Koger et al. (1970) found little measurable effect on ammonium acetate-extractable Ca below the 20 cm soil depth,

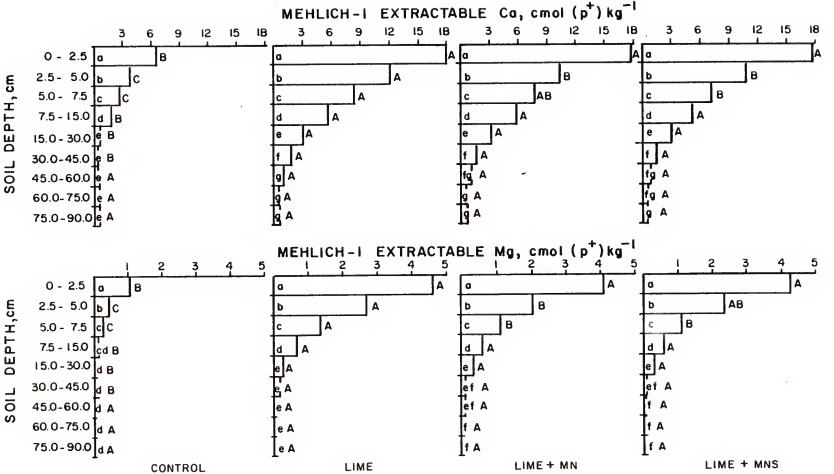

Fig. 6-5. Effects of lime-MN-S combinations and soil depth on Mehlich-I extractable Ca and Mg in the Myakka fine sand profile. Bars with the same lower-case letters within subfigures, and bars followed by the same upper-case letters within soil depth are indicative of no significant difference among means at the 5% level of probability (DMRT).

after 9 years of liming and phosphate fertilization on Myakka fine sand supporting grass-clover pastures. Blue (1979) reported, for the same plots as those sampled in this study, that unlimed soils had Mehlich-I extractable Ca of about 1 cmol (p^+) kg^{-1} soil in the top 15 cm of soil, after the first year of the sward establishment. The same unlimed plots 31 years later had extractable Ca of 6.5, 3.7, 2.7 and 1.7 cmol (p^+) kg^{-1} soil at the 0 to 2.5, 2.5 to 5.0, 5.0 to 7.5, and 7.5 to 15.0 cm soil depths. The build-up of extractable soil Ca over the years showed that Ca supply through periodic liming and annual application of superphosphate exceeded the amounts removed in forages and by the leaching process. Increases in soil organic matter content certainly provided the soil with more exchange sites, which contributed to cation retention. Extractable soil Mg decreased as the soil depth increased to 45 cm in limed soils and to 15 cm without lime. In the top layers of the soil profile, there was about 4 to 5 times more extractable Ca than Mg, whereas in deeper layers the ratio was increased to 10 to 12.

Although P fertilizer was applied equally to all plots, significant differences in Mehlich-I extractable soil P were observed for lime-Mn-S x soil depth combinations (Fig. 6-6). In the unlimed plots, the magnitude of the changes in extractable soil P with soil layers was smaller than in limed soils. Extractable P in the soil close to the surface (0 to 2.5 cm), and in the deepest layer sampled (75 to 90

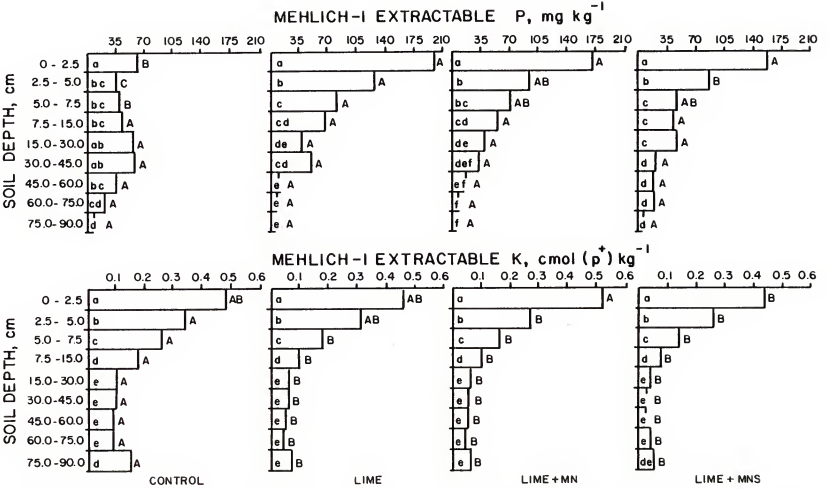

Fig. 6-6. Effects of lime-MN-S combinations and soil depth on Mehlich-I extractable P and K in the Myakka fine sand profile. Bars with the same lower-case letters within subfigures, and bars followed by the same upper-case letters within soil depth are indicative of no significant difference among means at the 5% level of probability (DMRT).

cm) was significantly different from that in most of the other soil layers analyzed. In the limed soils, decreases in extractable P occurred as the soil depth increased to 7.5 cm. Between soil depth of 7.5 and 45 cm, extractable P did not change appreciably, but it significantly decreased again at layers below 45 cm. Ozanne (1980) reported that P moves very slowly in the soil, and as a result, it tends to accumulate close to the soil surface. He also pointed out that the surface-applied P on swards and pastures would be retained in the surface 2 to 3 cm of soil. Barrow (1980) suggested that an accumulation of organic matter in the soil under permanent pastures may be accompanied by immobilization of P in the organic form. In the present study, organic matter was shown to accumulate in the soil, but that accumulation was not enhanced by lime applications. Part of the P accumulated in the surface layers of soil might be accounted for organic P. However, lime application was a major factor contributing to increased extractable P in the top 45 cm of soil, as well as resulting in a better distribution of extractable P in that part of the profile (Fig. 6-6). More P was retained in the soil where lime was applied. Neller et al. (1951a) found that liming prevented excessive leaching of P in Spodosols and that increasing soil pH to 5.7 resulted in retention of about 60% of the P applied in the top layers of such soils. When comparing lime-MN-S treatments at each soil depth, the limed plot without micronutrient additions showed significantly higher

values in the top three soil layers not only for extractable soil P (Fig. 6-6), but also for extractable soil Ca and Mg, than in the lime + MN or lime + MNS treatments. Blue (1979) found that micronutrients in addition to lime were the cause of significant increases in forage yields of white clover-Pensacola bahiagrass over a 25-year period in the experimental sampled area for the present study. The removal of P, Ca, and Mg might have been higher in forages from this treatment, with resulting depletion of their extractable amounts in the soil.

For Mehlich-I extractable soil K the interactions between lime-MN-S treatments x soil depth, sulfur application x soil depth, and lime-MN-S treatments x sulfur application were all statistically significant ($P<0.01$).

In examining the effects of lime-MN-S treatments at each soil depth, it was evident that, except for the two top soil layers, the unlimed soil always had higher extractable K than the limed plots (Fig. 6-6). At the 0 to 2.5 cm and 2.5 to 5.0 cm soil depths some limed plots could not be distinguished from the unlimed, in terms of the extractable soil K. Potassium fertilizer was applied at the same rates to the limed and unlimed plots, but forage yields and removal of the nutrient were much lower in the unlimed than in limed soils (Blue, 1979). In treatments with combinations of lime micronutrient, extractable soil K decreased with increasing soil depth to 15 to 30 cm. Probably the increased organic matter content in the top 30 cm of soil provided

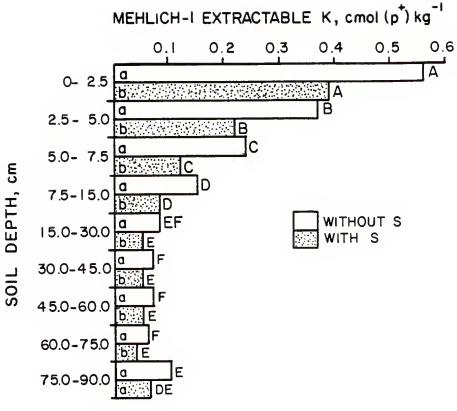

Fig. 6-7. Effects of sulfur fertilizations and soil depth on Mehlich-I extractable K in the Myakka fine sand profile. Bars with the same lower-case letters within soil depth, and bars followed by the same upper-case letters within S treatments are indicative of no significant difference among means at the 5% level of probability (DMRT).

sites where K was retained as exchangeable K. Below the top 30 cm of soil, and except the 75 to 90 cm soil depth in unlimed plots, no significant changes in extractable K occurred.

Without S fertilization, extractable soil K decreased with soil depth to 15 to 30 cm, then showed no significant changes down to 60 to 75 cm, and increased again in the 75 to 90 cm layer (Fig. 6-7). Where S fertilizers were applied, extractable K decreased with increasing soil depth to 15 to 30 cm, with no further changes as sampling depth increased to 90 cm. Within each soil depth studied, except for the 75 to 90 cm, extractable K was lower in the S fertilized subplots.

When S fertilization effects are considered within each lime-MN-S treatment, it is quite evident that extractable soil K decreased where S fertilizer was applied (Fig. 6-8). Data shown in Chapter III, experiment no. 1, and in Chapters IV and V in the present study indicate that extractable K in the soil was lowered in subplots with S fertilization. Extractable K was higher in the unlimed soil than in the limed soil, with or without S fertilization. Probably the lower forage yields in the unlimed soils resulted in lower K removal from the soil by the plants, and thus more K was left on the soil exchange sites.

Extractable soil Cu, Fe, Mn, and Zn as a function of the combinations between lime-MN-S treatments and soil depth

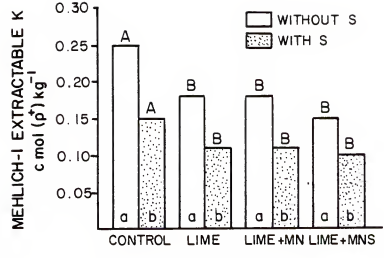

Fig. 6-8. Effects of lime-MN-S combinations and sulfur fertilizations on Mehlich-I extractable K in the Myakka fine sand. Bars with the same lower-case letters within lime-MN-S combinations, and bars followed by the same upper-case letters within S treatments are indicative of no significant difference among means at the 5% level of probability (DMRT).

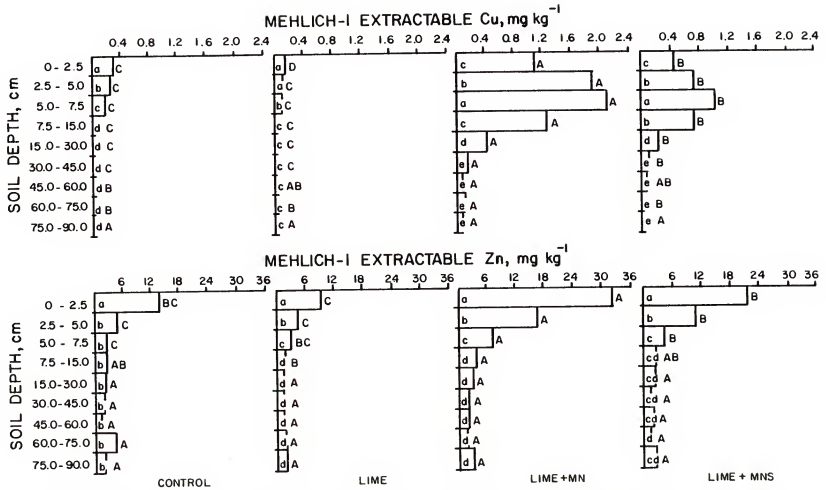

Fig. 6-9. Effects of lime-MN-S combinations and soil depth on Mehlich-I extractable Cu and Zn in the Myakka fine sand profile. Bars with the same lower-case letters within subfigures, and bars followed by the same upper-case letters within soil depth are indicative of no significant difference among means at the 5% level of probability (DMRT).

are presented in Fig. 6-9 and 6-10. Extractable Cu was significantly higher in the lime+MN and lime+MNS than in. the limed or control treatments at all except the 75 to 90 cm soil depth (Fig. 6-9). Soil under treatments lime+MN and lime+MNS received 16.8 kg Cu ha^{-1} from 1952 to 1959, and continued to show differences in extractable Cu 25 to 32 years later. Lime+MN treatment had higher extractable Cu than the lime+MNS. The lower extractable Cu in the lime+MNS treatment can not be attributed to higher plant uptake than in the lime+MN. Sulfur fertilization in 1980, 1981, and 1983/84 did not significantly affect extractable Cu. Detailed study of the lime-MN-S combinations x S fertilization interactions, indicated once more that extractable Cu was higher in soils with lime+MN than with lime+MNS, and that both of these treatments had higher extractable Cu than lime or control treatments. In treatments where micronutrients were not applied (control or lime), extractable Cu decreased with soil depth to 7.5 to 15 cm only. Below the 15 cm depth no significant changes occurred. However, under Cu fertilization (lime+MN and lime+MNS treatments), extractable Cu was higher in the top 30 cm of soil, with no further changes at deeper soil layers. The highest extractable Cu occurred at the 5.0 to 7.5 cm soil depth.

Extractable soil Zn was higher in the Zn-fertilized plots (lime+MN and lime+MNS treatments) than in the control or limed treatments, at the four soil layers in the top 15 cm of the profile. Below that soil depth and down to 90 cm,

no distinction could be made in extractable Zn between soil with or without applied Zn (Fig. 6-9). As for extractable Cu, extractable Zn was higher under lime+MN than lime+MNS. Similarly to extractable Cu, this can not be explained through plant uptake of Zn and removal from the area. Sulfur fertilization in 1980, 1981, and 1983/84 also resulted in a significant decrease in extractable Zn. The average extractable Zn in the subplots without S fertilizations was 5.2 mg kg^{-1} of soil, whereas in the S-fertilized soil, it was 4.5 mg kg^{-1} of soil. In limed soils, extractable Zn decreased as soil depth was increased to 15 cm. In the control treatment, only the 0 to 2.5 cm soil depth had more extractable Zn than the other soil layers sampled. In both lime+MN and lime+MNS, extractable Zn was highest in the 0 to 2.5 cm soil depth. Brown et al. (1962) applied $ZnSO_4$ to the surface of a soil placed in a column and passed water through it. They found that little movement of Zn occurred downward to 12.5 cm, and that most of the Zn was held in the 0 to 2.5 cm soil depth.

Extractable soil Mn was higher in limed soils than in the unlimed at any particular soil layer within the top 30 cm (Fig. 6-10). Below that point, no difference in extractable Mn between lime treatments could be found. Comparing the lime-MN-S combinations, the lime+MN treatment had higher extractable Mn than the lime treatment but only at the 0 to 2.5 cm soil depth. It is somewhat surprising to find higher extractable Mn in limed than in unlimed soils because Mn is

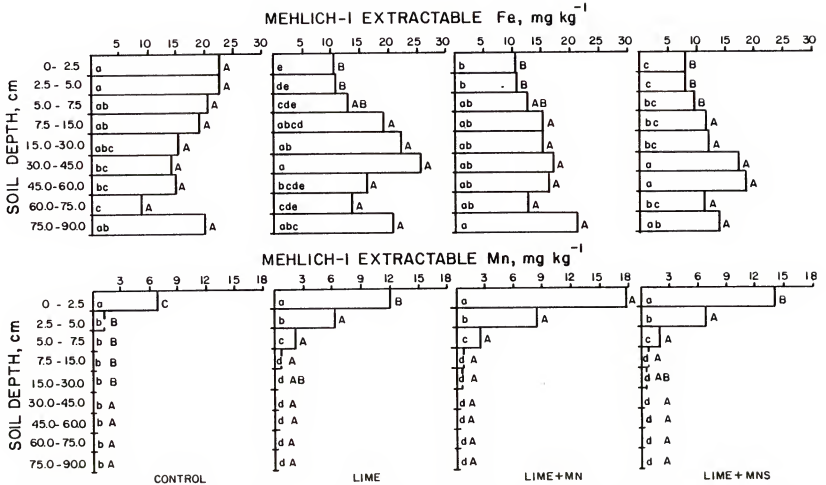

Fig. 6-10. Effects of lime-MN-S combinations and soil depth on Mehlich-I extractable Fe and Mn in the Myakka fine sand profile. Bars with the same lower-case letters within subfigures, and bars followed by the same upper-case letters within soil depth are indicative of no significant difference among means at the 5% level of probability (DMRT).

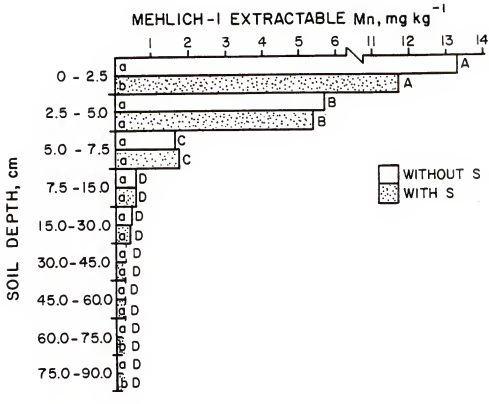

Fig. 6-11. Effects of sulfur fertilizations and soil depth on Mehlich-I extractable Mn in the Myakka fine sand profile. Bars with the same lower-case letters within soil depth and bars followed by the same upper-case letters within S treatments are indicative of no significant difference among means at the 5% level of probability (DMRT).

known to show markedly higher availability in soils with pH below 5.5 (Lucas and Knezek, 1972). As shown for extractable Zn in limed soils, extractable Mn decreased with increasing. soil depth down to 15 cm. In unlimed soil, extractable Mn accumulated only in the top 0 to 2.5 cm.

Detailed study of the S fertilization x soil depth interaction relating to extractable Mn showed that S application to the sward in 1980, 1981, and 1983/84 decreased extractable Mn at the 0 to 2.5 cm and 60 to 75 cm soil depths (Fig. 6-11). Extractable Mn decreased with increasing soil depth to 15 cm.

The distribution of extractable soil Fe in the profile was much different than that for extractable soil Cu, Mn, and Zn. Downward movement of organic Fe complexes may have been the reason for the observed distribution of extractable Fe in the profile. In contrast to unlimed soil, extractable Fe in limed treatments increased as soil depth increased from 5 to 45 or 60 cm (Fig.6-10). In several samples from limed soils, higher extractable Fe was found in the 30 to 45 cm and 45 to 60 cm soil depth. In the control treatment, lower extractable Fe than in the top 5 cm of soil was found only at the 30 to 45 cm, 45 to 60 cm, and 60 to 75 cm soil depths. At any soil depth below 7.5 cm, the four lime-MN-S combinations could not be differentiated in terms of extractable Fe. In the top 5 cm of soil, extractable Fe was higher in the control than in the lime, lime+MN, or lime+MNS treatments.

Sulfur application to the sward in 1980, 1981, and 1983/84 resulted in significantly higher extractable Fe in the soil sampled in 1984. Soil samples collected from subplots fertilized with S had average extractable Fe of 16.1 mg kg^{-1} of soil, whereas subplots without S application had 14.3 mg kg^{-1} of soil.

Summary

Soil profile samples to a depth of 90 cm were taken in 1984 from a Spodosol which had received differential fertilizer and lime applications, and had supported a mixed sward of white clover and Pensacola bahiagrass since 1952. The objective was to evaluate the effects of lime, micronutrients, and S applications on changes and distribution of extractable nutrients, pH, and organic carbon at nine soil depths. Soil was sampled in the following plots: control, lime (from 1952 to 1983 liming in a total amount of 15 Mg ha^{-1}), lime+MN (besides lime, Cu, Fe, Mn, and Zn were added from 1952 to 1959 in total amounts of 16.8, 3.2, 4.6, and 15.6 kg ha^{-1}, respectively), and lime+MNS (besides lime+MN, B was applied from 1952 to 1959 in a total amount of 4.8 kg ha^{-1}, and 30 kg S ha^{-1} were added in 1982). Two subplots in each lime-MN-S combination with and without S fertilization in 1980, 1981, and 1983/84 at a total rate of 200 kg ha^{-1}. Phosphorus and K were applied annually to all treatments and subplots. White clover was present only in limed plots. Total N and organic C decreased with soil depth

in the surface 30 and 45 cm, respectively. Limed soils had higher total N in the top 7.5 cm of soil, and higher pH, and Mehlich-I extractable Ca and Mg in the top 45 cm of soil, than control plots. Extractable Ca and Mg decreased with increase in soil depth to 45 cm in limed plots. Extractable P was higher in limed soils, and was retained in the top 7.5 cm of soil. Extractable K was lower in limed plots and in S-treated subplots than in unlimed soils or non-S-treated subplots, and decreased with soil depth to 30 cm. Sulfur fertilization in increased phosphate extractable S in the 15 cm of surface soil and at the 75 to 90 cm soil depth, but did not affect total S in the soil. Mehlich-I extractable Cu and Zn were markedly higher in soils fertilized with these micronutrients 25 to 32 years before soil sampling. Where Cu-containing fertilizer was applied, extractable Cu decreased with soil depth to 45 cm, and to 15 cm without Cu fertilization. Both extractable Mn and Zn decreased as soil depth increased to 15 cm in limed soils, and were higher at the 0 to 2.5 cm soil depth in unlimed soil. Extractable Fe distribution in the profile had different pattern than those for extractable Cu, Mn, and Zn. In large a number of samples, extractable Fe was higher at 30 to 45 and 45 to 60 cm soil depth.

CHAPTER VII
CONCLUSIONS

Data obtained in a series of field and greenhouse experiments with a Spodosol which has supported white clover-Pensacola bahiagrass swards for 28 to 32 years, warrant the following conclusions:

1. Sulfur fertilization increased forage yield, and N and S contents in the mixed sward every year during the 4 years of the field experiment with Sward I, and during 1 year with Sward II.

2. Sulfur fertilization superimposed in the field on soil with or without previous S fertilization increased forage yield and S content in the mixed sward in the field, but was not beneficial white clover grown in pots.

3. A single S application early in the winter with low rainfall or split S applications throughout the winter with a substantial amount of rain resulted in more benefit for white clover in the mixed sward than a single application during winter with high rainfall.

4. Even under S fertilization, white clover grown in mixture with the bahiagrass had a S concentration and N:S ratio in the S-deficient range. Pensacola bahiagrass had S concentrations higher and N:S ratios lower than the adequate

range, which indicated that N supply was limiting higher productivity of the grass.

5. The apparent S recovery from the fertilizer added in the field was 13.2% over the 4-year period when 200 kg S ha^{-1} were applied to Sward I, and 7.3 and 4.8%, when the respective S rates were 45 and 90 kg ha^{-1} in the 1-year study with Sward II. Low S recovery and soil analysis data suggested that most of the applied S was leached out of the 0 to 90 cm soil depth. In the greenhouse, where no leaching was allowed, the apparent plant S recovery ranged within 51 to 53% and 30 to 33% at S rates of 22.5 and 45.0 mg kg^{-1} of soil, respectively. About 20% more of the S was recovered in the soil, but part of the applied S was not accounted for by plant uptake and the total soil S.

6. Lower K concentrations in white clover herbage and stolon-root tissues of the grass were recorded after S applications in the field.

7. Sulfur applications to the soil in pots increased forage yield, N and S concentrations, and N and S contents in white clover, but decreased the N:S ratio of the legume irrespective of previous S treatment to the soil. Plants developed under no current S application had shorter stolons, reduced tillering, fewer flower heads, and reduced number and size of nodules than plants in the S-treated pots. Sulfur-deficient plants had yellowish leaflets and reddened petioles.

8. Normal white clover plants, with adequate S supply had N:S ratios in the petioles + leaflets ranging within 16.3:1 to 18.3:1, whereas values exceeding 19:1 were found under no S applications.

9. The application of 45 mg S kg^{-1} of soil as gypsum or potassium sulfate appeared to provide sufficient S for white clover growth in the greenhouse when plants had taken up ample amounts of K. Plants with low K concentrations always had S concentrations and N:S ratios in the deficient range. Applications of K at 75 mg kg^{-1} of soil for one growth or 110 and 220 mg kg^{-1} of soil for two growth cycles of white clover resulted in low K and low S concentrations in the legume.

10. Gypsum and potassium sulfate proved to have similar efficiency when applied to the Spodosol used in the greenhouse, provided that K was added to the soil at equivalent rates.

11. White clover plants grown on soil that received 220 mg K kg^{-1} of soil had higher stolon and root yields, higher K concentrations and contents, and lower Ca and Mg concentrations than those under 110 mg K kg^{-1} of soil.

12. Concentration of Mo in white clover grown in pots or in the mixed sward was decreased by the S applications, but was increased by Mo fertilization.

13. Stolon-root tissue of Pensacola bahiagrass sampled in the field provided no evidence of S storage in those plant tissues during the spring.

14. Extractable soil S was increased by S fertilization both in the pots and in the field.

15. Limed soil, which had white clover present, had higher total N in the top 7.5 cm of than unlimed soil.

16. Limed plots had higher soil pH, and higher extractable soil Ca and Mg than unlimed plots, at any soil layer in the top 45 cm of soil.

17. Soil organic C, total N, total S, and extractable Ca, Mg, and K decreased with increasing soil depth to 30 or 45 cm, with no further changes to 90 cm.

18. Soil extractable P was higher in limed plots, and was mainly retained in the surface 7.5 cm of soil.

19. Lower extractable K was found in the S-fertilized soil in the greenhouse and in the field. Extractable K was lower at each soil depth to 90 cm in the profile of the S-fertilized plots. Unlimed soil had higher extractable K than the limed soil.

20. Extractable Cu and Zn were markedly higher in the soil that received micronutrient fertilizations 25 to 32 years before soil sampling.

21. Extractable soil Cu, Mn, and Zn were more uniformly distributed in the profile of limed soil than unlimed soil. Extractable Fe was distributed in the profile in a different pattern than Cu, Mn, and Zn, and was higher at 30 to 45 cm and 45 to 60 cm soil depth in a large number of samples.

22. Under conditions similar to those found in this study, S fertilization is a major requirement for white

clover growth in the Spodosol. Further research on the relationship between S fertilization and lowering in the extractable K in the soil is suggested.

APPENDIX
NUMERICAL DATA ON CHANGES AND DISTRIBUTION OF EXTRACTABLE NUTRIENTS, pH, AND ORGANIC CARBON IN A SPODOSOL PROFILE.

Table A-1. Distribution of organic C, and C:N and C:S ratios in the Myakka fine sand profile.

Soil depth	Soil		
	Organic C	C:N	C:S
cm	g kg^{-1}	--------ratio--------	
0 - 2.5	62.0a*	20.4d	139b
2.5 - 5.0	41.4b	23.3d	164b
5.0 - 7.5	29.6c	28.8cd	218b
7.5 - 15.0	18.4d	29.3cd	231b
15.0 - 30.0	15.7de	42.0bc	389a
30.0 - 45.0	12.8ef	42.8bc	384a
45.0 - 60.0	11.8ef	50.4ab	387a
60.0 - 75.0	10.6ef	61.6a	391a
75.0 - 90.0	9.1f	50.2ab	192b

*Means within columns followed by the same letters are not significantly different at the 5% level of probability (DMRT).

Table A-2. Effects of lime-MN-S combinations and soil depth on total N and total S in the Myakka fine sand profile.

Soil depth	Lime-MN-S combinations#					
	Control	L@	L+MNS	Control	L	L+MNS
cm	Total N, mg kg^{-1}			Total S, mg kg^{-1}		
0- 2.5	2399^{B*}_{a}	3336^{A}_{a}	3341^{A}_{a}	381^{B}_{a}	475^{A}_{a}	458^{A}_{a}
2.5- 5.0	1472^{B}_{b}	2038^{A}_{b}	1826^{A}_{b}	229^{A}_{b}	288^{A}_{b}	243^{A}_{b}
5.0- 7.5	935^{B}_{c}	1233^{A}_{c}	1147^{A}_{c}	138^{A}_{c}	164^{A}_{c}	132^{A}_{c}
7.5-15.0	547^{A}_{d}	613^{A}_{d}	583^{A}_{d}	72^{AB}_{d}	94^{A}_{d}	65^{B}_{d}
15.0-30.0	327^{A}_{e}	343^{A}_{e}	370^{A}_{e}	58^{A}_{d}	34^{A}_{e}	37^{A}_{e}
30.0-45.0	339^{A}_{e}	275^{A}_{e}	310^{A}_{e}	46^{A}_{d}	32^{A}_{e}	35^{A}_{e}
45.0-60.0	266^{A}_{e}	231^{A}_{e}	277^{A}_{e}	40^{A}_{d}	32^{A}_{e}	34^{A}_{e}
60.0-75.0	183^{A}_{e}	160^{A}_{e}	196^{A}_{e}	66^{A}_{d}	23^{A}_{e}	31^{A}_{e}
75.0-90.0	194^{A}_{e}	158^{A}_{e}	178^{A}_{e}	56^{A}_{d}	41^{A}_{e}	45^{A}_{de}

#Fertilizer treatments applied to the clover-grass sward from 1952 to 1984.
@Stands for Lime.
*Data within subtables for each soil depth over the three lime-MN-S combinations followed by the same upper-case letters, and for each column followed by the same lower-case letters are not significantly different at the 5% level of probability (DMRT).

Table A-3. Effects of lime-MN-S combinations and soil depth on soil pH in water and in 1 M KCl in the Myakka fine sand profile.

Soil depth	Lime-Mn-S combinations#							
	Cont.	L@	L+MN	L+MNS	Cont.	L	L+MN	L+MNS
cm	Soil pH in water				Soil pH in 1 M KCl			
0- 2.5	4.8^{B*}_{cd}	6.1^{A}_{a}	6.0^{A}_{a}	6.1^{A}_{a}	4.0^{B}_{b}	5.7^{A}_{a}	5.5^{A}_{a}	5.6^{A}_{a}
2.5- 5.0	4.7^{B}_{d}	6.1^{A}_{a}	6.0^{A}_{a}	6.0^{A}_{a}	3.6^{B}_{cd}	5.5^{A}_{b}	5.4^{A}_{ab}	5.5^{A}_{a}
5.0- 7.5	4.7^{C}_{d}	6.0^{A}_{ab}	5.8^{B}_{ab}	5.8^{B}_{b}	3.6^{C}_{cd}	5.4^{A}_{b}	5.2^{B}_{bc}	5.1^{B}_{b}
7.5-15.0	4.8^{C}_{d}	6.0^{A}_{ab}	5.8^{B}_{ab}	5.7^{B}_{bc}	3.6^{C}_{cd}	5.4^{A}_{b}	5.1^{B}_{c}	5.0^{B}_{b}
15.0-30.0	4.9^{C}_{bc}	6.1^{A}_{a}	5.9^{B}_{a}	5.8^{B}_{b}	3.7^{C}_{c}	5.4^{A}_{b}	5.1^{B}_{c}	5.0^{B}_{b}
30.0-45.0	4.9^{C}_{b}	5.8^{A}_{b}	5.5^{B}_{bc}	5.5^{B}_{c}	3.9^{C}_{b}	5.0^{A}_{c}	4.7^{B}_{d}	4.7^{AB}_{c}
45.0-60.0	5.1^{B}_{a}	5.6^{A}_{c}	5.4^{A}_{c}	5.4^{A}_{d}	4.2^{A}_{a}	4.7^{A}_{d}	4.6^{A}_{d}	4.5^{A}_{d}
60.0-75.0	5.2^{A}_{a}	5.4^{A}_{c}	5.3^{A}_{cd}	5.3^{A}_{d}	4.4^{A}_{a}	4.6^{A}_{d}	4.5^{A}_{d}	4.5^{A}_{d}
75.0-90.0	5.1^{A}_{a}	5.1^{A}_{d}	5.1^{A}_{d}	5.0^{A}_{e}	4.4^{A}_{a}	4.4^{A}_{e}	4.4^{A}_{d}	4.4^{A}_{d}

#Fertilizer treatments applied to the clover-grass sward from 1952 to 1984.
@Stands for Lime.
*Data within subtables for each soil depth over the four lime-MN-S combinations followed by the same upper-case letters, and for each column followed by the same lower-case letters are not significantly different at the 5% level of probability (DMRT).

Table A-4. Effects of lime-MN-S combinations and soil depth on Mehlich-I extractable Ca and Mg in the Myakka fine sand profile.

Soil depth	Lime-Mn-S combinations#							
	Cont.	L@	L+MN	L+MNS	Cont.	L	L+MN	L+MNS
cm	Ext.Φ Ca, cmol(p+)kg^{-1}				Ext. Mg, cmol(p+)kg^{-1}			
0- 2.5	6.5^{B*}_{a}	18.1^{A}_{a}	17.6^{A}_{a}	17.7^{A}_{a}	1.03^{B}_{a}	4.57^{A}_{a}	4.12^{A}_{a}	4.27^{A}_{a}
2.5- 5.0	3.7^{C}_{b}	12.1^{A}_{b}	10.3^{B}_{b}	10.9^{B}_{b}	0.44^{C}_{b}	2.64^{A}_{b}	2.05^{B}_{b}	2.33^{AB}_{b}
5.0- 7.5	2.7^{C}_{c}	8.4^{A}_{c}	7.6^{AB}_{c}	7.1^{B}_{c}	0.23^{C}_{c}	1.37^{A}_{c}	1.10^{B}_{c}	1.10^{B}_{c}
7.5-15.0	1.7^{B}_{d}	5.7^{A}_{d}	5.7^{A}_{d}	5.3^{A}_{d}	0.12^{B}_{cd}	0.64^{A}_{d}	0.57^{A}_{d}	0.56^{A}_{d}
15.0-30.0	0.7^{B}_{e}	3.0^{A}_{e}	2.9^{A}_{e}	2.9^{A}_{e}	0.06^{B}_{d}	0.29^{A}_{e}	0.31^{A}_{e}	0.29^{A}_{e}
30.0-45.0	0.5^{B}_{e}	1.9^{A}_{f}	1.6^{A}_{f}	1.4^{A}_{f}	0.04^{B}_{d}	0.14^{A}_{e}	0.13^{A}_{ef}	0.12^{A}_{ef}
45.0-60.0	0.4^{A}_{e}	0.9^{A}_{g}	1.1^{A}_{fg}	0.8^{A}_{fg}	0.03^{A}_{d}	0.06^{A}_{e}	0.13^{A}_{ef}	0.06^{A}_{f}
60.0-75.0	0.3^{A}_{e}	0.5^{A}_{g}	0.5^{A}_{g}	0.6^{A}_{fg}	0.03^{A}_{d}	0.04^{A}_{e}	0.04^{A}_{f}	0.05^{A}_{f}
75.0-90.0	0.5^{A}_{e}	0.6^{A}_{g}	0.6^{A}_{g}	0.5^{A}_{g}	0.04^{A}_{d}	0.05^{A}_{e}	0.05^{A}_{f}	0.04^{A}_{f}

#Fertilizer treatments applied to the clover-grass sward from 1952 to 1984.
@Stands for Lime.
ΦStands for Extractable.
*Data within subtables for each soil depth over the four lime-MN-S combinations followed by the same upper-case letters, and for each column followed by the same lower-case letters are not significantly different at the 5% level of probability (DMRT).

Table A-5. Effects of lime-MN-S combinations and soil depth on Mehlich-I extractable K and P in the Myakka fine sand profile.

Soil depth	Lime-Mn-S combinations#							
	Cont.	L@	L+MN	L+MNS	Cont.	L	L+MN	L+MNS
cm	Ext.Φ K, cmol(p^+)kg^{-1}				Ext. P, mg kg^{-1}			
0- 2.5	0.48^{AB*}_{a}	0.46^{AB}_{a}	0.52^{A}_{a}	0.44^{B}_{a}	60^{B}_{a}	199^{A}_{a}	171^{A}_{a}	156^{A}_{a}
2.5- 5.0	0.34^{A}_{b}	0.31^{AB}_{b}	0.27^{B}_{b}	0.26^{B}_{b}	34^{C}_{bc}	127^{A}_{b}	93^{AB}_{b}	85^{B}_{b}
5.0- 7.5	0.26^{A}_{c}	0.18^{B}_{c}	0.16^{B}_{c}	0.14^{B}_{c}	38^{B}_{bc}	81^{A}_{c}	72^{AB}_{bc}	46^{AB}_{c}
7.5-15.0	0.18^{A}_{d}	0.10^{B}_{d}	0.10^{B}_{d}	0.08^{B}_{d}	41^{A}_{bc}	65^{A}_{cd}	54^{A}_{cd}	42^{A}_{c}
15.0-30.0	0.10^{A}_{e}	0.06^{B}_{e}	0.06^{B}_{e}	0.04^{B}_{e}	54^{A}_{ab}	37^{A}_{de}	38^{A}_{de}	45^{A}_{c}
30.0-45.0	0.10^{A}_{e}	0.06^{B}_{e}	0.05^{B}_{e}	0.03^{B}_{e}	57^{A}_{ab}	50^{A}_{cd}	32^{A}_{def}	21^{A}_{d}
45.0-60.0	0.09^{A}_{e}	0.05^{B}_{e}	0.05^{B}_{e}	0.03^{B}_{e}	34^{A}_{bc}	11^{A}_{e}	16^{A}_{ef}	16^{A}_{d}
60.0-75.0	0.09^{A}_{e}	0.04^{B}_{e}	0.04^{B}_{e}	0.04^{B}_{e}	21^{A}_{cd}	6^{A}_{e}	7^{A}_{f}	18^{A}_{d}
75.0-90.0	0.15^{A}_{d}	0.07^{B}_{e}	0.06^{B}_{e}	0.05^{B}_{de}	6^{A}_{d}	4^{A}_{e}	4^{A}_{f}	5^{A}_{d}

#Fertilizer treatments applied to the clover-grass sward from 1952 to 1984.
@Stands for Lime.
ΦStands for Extractable.
*Data within subtables for each soil depth over the four lime-MN-S combinations followed by the same upper-case letters, and for each column followed by the same lower-case letters are not significantly different at the 5% level of probability (DMRT).

Table A-6. Effects of lime-MN-S combinations and soil depth on Mehlich-I extractable Cu and Zn in the Myakka fine sand profile.

Soil depth	Lime-Mn-S combinations#							
	Cont.	L@	L+MN	L+MNS	Cont.	L	L+MN	L+MNS
cm	Ext.Φ Cu, mg kg^{-1}				Ext. Zn, mg kg^{-1}			
0- 2.5	0.28^{C*}_{a}	0.13^{D}_{a}	1.09^{A}_{c}	0.43^{B}_{c}	13.5^{BC}_{a}	9.0^{C}_{a}	31.9^{A}_{a}	21.5^{B}_{a}
2.5- 5.0	0.24^{C}_{b}	0.12^{C}_{a}	1.89^{A}_{b}	0.71^{B}_{b}	4.5^{C}_{b}	4.2^{C}_{b}	16.1^{A}_{b}	10.5^{B}_{b}
5.0- 7.5	0.15^{C}_{c}	0.09^{C}_{b}	2.09^{A}_{a}	1.01^{B}_{a}	2.2^{C}_{b}	2.7^{BC}_{c}	6.9^{A}_{c}	4.0^{B}_{c}
7.5-15.0	0.08^{C}_{d}	0.06^{C}_{c}	1.25^{A}_{c}	0.73^{B}_{b}	2.2^{AB}_{b}	1.4^{B}_{d}	3.4^{A}_{d}	2.2^{AB}_{cd}
15.0-30.0	0.04^{C}_{d}	0.05^{C}_{c}	0.41^{A}_{d}	0.23^{B}_{d}	2.0^{A}_{b}	1.2^{A}_{d}	2.9^{A}_{d}	2.4^{A}_{cd}
30.0-45.0	0.05^{C}_{d}	0.05^{C}_{c}	0.16^{A}_{e}	0.10^{B}_{e}	1.6^{A}_{b}	1.2^{A}_{d}	1.9^{A}_{d}	1.6^{A}_{cd}
45.0-60.0	0.05^{B}_{d}	0.06^{AB}_{c}	0.09^{A}_{e}	0.08^{AB}_{e}	1.3^{A}_{b}	1.2^{A}_{d}	1.8^{A}_{d}	1.8^{A}_{cd}
60.0-75.0	0.04^{B}_{d}	0.04^{B}_{c}	0.12^{A}_{e}	0.06^{B}_{e}	4.1^{A}_{b}	1.4^{A}_{d}	1.5^{A}_{d}	1.3^{A}_{d}
75.0-90.0	0.05^{A}_{d}	0.06^{A}_{c}	0.08^{A}_{e}	0.06^{A}_{e}	1.6^{A}_{b}	1.6^{A}_{d}	2.9^{A}_{d}	2.4^{A}_{cd}

#Fertilizer treatments applied to the clover-grass sward from 1952 to 1984.
@Stands for Lime.
ΦStands for Extractable.
*Data within subtables for each soil depth over the four lime-MN-S combinations followed by the same upper-case letters, and for each column followed by the same lower-case letters are not significantly different at the 5% level of probability (DMRT).

Table A-7. Effects of lime-MN-S combinations and soil depth on Mehlich-I extractable Fe and Mn in the Myakka fine sand profile.

Soil depth	Lime-Mn-S combinations#							
	Cont.	L@	L+MN	L+MNS	Cont.	L	L+MN	L+MNS
cm	Ext.Φ Fe, mg kg^{-1}				Ext. Mn, mg kg^{-1}			
0- 2.5	22.5^{A*}_{a}	10.4^{B}_{e}	10.6^{B}_{b}	7.9^{B}_{c}	6.62^{C}_{a}	11.88^{B}_{a}	17.75^{A}_{a}	13.69^{B}_{a}
2.5- 5.0	22.4^{A}_{a}	10.7^{B}_{de}	10.7^{B}_{b}	7.8^{B}_{c}	0.90^{B}_{b}	6.38^{A}_{b}	8.36^{A}_{b}	6.56^{A}_{b}
5.0- 7.5	20.6^{A}_{ab}	12.8^{AB}_{cde}	12.6^{AB}_{ab}	9.4^{B}_{bc}	0.36^{B}_{b}	2.20^{A}_{c}	2.45^{A}_{c}	1.78^{A}_{c}
7.5-15.0	18.9^{A}_{ab}	18.8^{A}_{abcd}	15.1^{A}_{ab}	11.6^{A}_{bc}	0.19^{B}_{b}	0.63^{A}_{d}	0.73^{A}_{d}	0.54^{A}_{d}
15.0-30.0	15.2^{A}_{abc}	22.0^{A}_{ab}	15.1^{A}_{ab}	11.9^{A}_{bc}	0.19^{B}_{b}	0.39^{AB}_{d}	0.65^{A}_{d}	0.43^{AB}_{d}
30.0-45.0	14.2^{A}_{bc}	25.4^{A}_{a}	16.8^{A}_{ab}	17.2^{A}_{a}	0.24^{A}_{b}	0.24^{A}_{d}	0.27^{A}_{d}	0.26^{A}_{d}
45.0-60.0	14.7^{A}_{bc}	16.2^{A}_{bcde}	16.1^{A}_{ab}	18.5^{A}_{a}	0.24^{A}_{b}	0.19^{A}_{d}	0.21^{A}_{d}	0.22^{A}_{d}
60.0-75.0	8.8^{A}_{c}	13.6^{A}_{cde}	12.7^{A}_{ab}	11.2^{A}_{bc}	0.16^{A}_{b}	0.13^{A}_{d}	0.14^{A}_{d}	0.17^{A}_{d}
75.0-90.0	19.8^{A}_{ab}	20.6^{A}_{abc}	20.9^{A}_{a}	13.8^{A}_{ab}	0.15^{A}_{b}	0.14^{A}_{d}	0.16^{A}_{d}	0.14^{A}_{d}

#Fertilizer treatments applied to the clover-grass sward from 1952 to 1984.
@Stands for Lime.
*Data within subtables for each soil depth over the four lime-MN-S combinations followed by the same upper-case letters, and for each column followed by the same lower-case letters are not significantly different at the 5% level of probability (DMRT).

Table A-8. Effects of sulfur fertilization and soil depth on phosphate-extractable S, and on Mehlich-I extractable K and Mn in the Myakka fine sand profile.

Soil depth	Sulfur fertilization					
	No	Yes#	No	Yes#	No	Yes#
	Extractable S		Extractable K		Extractable Mn	
cm	--mg kg^{-1}--		cmol(p^+)kg^{-1}		----mg kg^{-1}----	
0- 2.5	4.2^{B*}_{b}	5.4^{A}_{b}	0.56^{A}_{a}	0.39^{B}_{a}	13.29^{A}_{a}	11.68^{B}_{a}
2.5- 5.0	2.9^{A}_{cd}	3.2^{A}_{cde}	0.37^{A}_{b}	0.22^{B}_{b}	5.70^{A}_{b}	5.40^{A}_{b}
5.0- 7.5	1.9^{B}_{ef}	2.3^{A}_{cde}	0.24^{A}_{c}	0.12^{B}_{c}	1.66^{A}_{c}	1.73^{A}_{c}
7.5-15.0	1.4^{B}_{f}	1.7^{A}_{e}	0.15^{A}_{d}	0.08^{B}_{d}	0.53^{A}_{d}	0.52^{A}_{d}
15.0-30.0	1.6^{A}_{f}	2.0^{A}_{de}	0.08^{A}_{ef}	0.05^{B}_{e}	0.44^{A}_{d}	0.38^{A}_{d}
30.0-45.0	2.6^{A}_{de}	3.0^{A}_{cde}	0.07^{A}_{f}	0.05^{B}_{e}	0.26^{A}_{d}	0.24^{A}_{d}
45.0-60.0	3.1^{A}_{cd}	3.5^{A}_{cd}	0.07^{A}_{f}	0.05^{B}_{e}	0.23^{A}_{d}	0.21^{A}_{d}
60.0-75.0	3.6^{A}_{bc}	3.9^{A}_{bc}	0.06^{A}_{f}	0.04^{B}_{e}	0.17^{A}_{d}	0.13^{B}_{d}
75.0-90.0	7.5^{B}_{a}	11.0^{A}_{a}	0.10^{A}_{e}	0.06^{A}_{de}	0.17^{A}_{d}	0.13^{B}_{d}

#Sulfur applied to the clover-grass sward in 1980, 1981 and 1983/84, at a total rate of 200 kg ha^{-1}.

*Data within subtables for each soil depth over the two sulfur treatments (No or Yes) followed by the same upper-case letters, and for each column followed by the same lower-case letters are not significantly different at the 5% level of probability (DMRT).

REFERENCES

Adams, A. F. R. 1973. Sulphur on New Zealand pastures--effect of rates and form. Sulphur Inst. J. 9(2):14-16.

Adams, W. E., R. W. Pearson, W. A. Jackson, and R. A. McCreery. 1967. Influence of limestone and nitrogen on soil pH and Coastal bermudagrass yield. Agron. J. 59:450-453.

Alexander, M. 1977. Introduction to soil microbiology. 2nd ed. John Wiley and Sons, New York.

Anderson, A. J. 1956. Molybdenum deficiencies in legumes in Australia. Soil Sci. 81:173-182.

Anderson, A. J., and D. Spencer. 1950. Sulphur in nitrogen metabolism of legumes and non-legumes. Aust. J. Sci. Res. 3:431-449.

Anderson, G. 1975. Sulfur in soil organic substances. p. 333-341. In J. E. Gieseking (ed.) Soil components, Vol. 1. Springer-Verlag, New York.

Andrew, C. S. 1977. The effect of sulfur on the growth, sulphur and nitrogen concentrations, and critical sulphur concentrations of some tropical and temperate pasture legumes. Aust. J. Agric. Res. 28:807-820.

Arkley, T. H. 1961. Sulfur compounds in soil systems. Ph.D. diss. Univ. of California, Berkeley.

Atlas, R. M., and R. Bartha. 1981. Microbial Ecology: Fundamentals and applications. Allison-Wesley Publishing Co., Reading, MA.

Baltensperger, D. D., C. E. Dean, E. S. Horner, L. S. Dunavin, W. R. Ocumpaugh, and P. Mislevy. 1984. 'Osceola' white clover. Fla. Agric. Exp. Stn. Circular S-311.

Barber, S. A. 1962. A diffusion and mass-flow concept of soil nutrient availability. Soil Sci. 93:39-49.

Bardsley, C. E. 1960. Absorption of sulfur from organic and inorganic sources by bush beans. Agron. J. 52:485-486.

Bardsley, C. E., Jr., and H. V. Jordan. 1957. Sulfur availability in seven southeastern soils as measured by growth and composition of white clover. Agron. J. 49:310-312.

Bardsley, C. E., and V. J. Kilmer. 1963. Sulfur supply of soils and crop yields in the Southeastern United States. Soil Sci. Soc. Am. Proc. 27:197-199.

Bardsley, C. E., and J. D. Lancaster. 1960. Determination of reserve sulfur and soluble sulfates in soils. Soil Sci. Soc. Am. Proc. 24:265-268.

Barrow, N. J. 1966. The residual value of phosphorus and sulphur components of superphosphate on some Western Australian soils. Aust. J. Exp. Agric. Anim. Husb. 6:9-16.

Barrow, N. L. 1967. Studies on extraction and on availability to plants of adsorbed plus soluble sulfate. Soil Sci. 104:242-249.

Barrow, N. J. 1980. Evaluation and utilization of residual phosphorus in soils. p. 333-359. In F. E. Khasawneh, E. C. Sample, and E. J. Kamprath (ed.) The role of phosphorus in agriculture. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Beaton, J. D., G. R. Burns, and J. Platou. 1968. Determination of sulphur in soils and plant material. Tech. bull. no. 14, The Sulphur Institute, Washington, D. C.

Bettany, J. R., J. W. B. Stewart, and E. H. Halstead. 1973. Sulfur fractions and carbon, nitrogen, and sulfur relationships in grassland, forest, and associated transitional soils. Soil Sci. Soc. Am. Proc. 37:915-918.

Bingley, J. B. 1963. Determination of molybdenum in biological materials with dithiol. Control of copper interference. J. Agric. Food Chem. 11:130-131.

Bledsoe, R. W., and R. E. Blaser. 1947. The influence of sulfur on the yield and composition of clovers fertilized with different sources of phosphorus. J. Am. Soc. Agron. 39:146-152.

Blue, W. G. 1970. The effect of lime on retention of fertilizer phosphorus in Leon fine sand. Proc. Soil Crop Sci. Soc. Fla. 30:141-150.

Blue, W. G. 1979. Forage production and N contents, and soil changes during 25 years of continuous white clover-Pensacola bahiagrass growth on a Florida Spodosol. Agron. J. 71:795-798.

Blue, W. G., and V. W. Carlisle. 1985. Soils for clovers. In N. L. Taylor (ed.) Clover science and technology.- Agronomy 25:185-204. Am. Soc. Agron., Crop Sci. Soc. Am., Madison, WI.

Blue, W. G., N. Gammon, Jr., and H. W. Winsor. 1964. Accumulation of organic matter and nitrogen in flatwoods soils planted to white clover-grass pastures. Proc. Soil Crop Sci. Soc. Fla. 24:20-26.

Blue, W. G., and D. A. Graetz. 1977. The effect of split nitrogen applications on nitrogen uptake by Pensacola bahiagrass from an Aeric Haplaquod. Soil Sci. Soc. Am. J. 41:927-930.

Blumenthal, P. L., and S. C. Guernsey. 1915. The determination of sulfur as barium sulfate. Iowa Agric. Exp. Stn. Res. Bull. 26.

Brezonik, P. L., E. S. Edgerton, and C. D. Hendry. 1980. Acid precipitation and sulfate deposition in Florida. Science. 208:1027-1029.

Brown, A. L., B. A. Krantz, and P. E. Martin. 1962. Plant uptake and fate of soil-applied zinc. Soil Sci. Soc. Am. Proc. 26:167-170.

Brown, G., and R. Kanaris-Sotiriou. 1969. The determination of sulphur in soils by X-ray fluorescence analysis. Analyst 94:782-786.

Bryan, O. C. 1958. Soils of Florida and their crop adaptation. Department of Agriculture, State of Florida, Bull. No. 42.

Bryan, W. W., and T. R. Evans. 1971. Grazing trials on the Wallum of south-eastern Queensland. 3. A nursery grazed by sheep. Aust. J. Exp. Agric. Anim. Husb. 11:633-639.

Buol, S. W., F. D. Hole, and R. J. McCracken. 1980. Soil genesis and classification. 2nd ed., Iowa State University Press, Ames, IA.

Butters, B., and E. M. Chenery. 1959. A rapid method for the determination of total sulphur in soils and plants. Analyst. 84:239-245.

Cairns, R. R., and R. B. Carson. 1961. Effect of sulphur treatments on yield and nitrogen and sulphur content of alfalfa grown on sulphur-deficient and sulphur-sufficient grey wooded soils. Can. J. Plant Sci. 41:709-715.

Carlisle, V. W., and R. B. Brown. 1982. Florida soil identification handbook. Soil Science Department, University of Florida, Gainesville, Florida.

Chambliss, C. G., and D. W. Jones. 1981. Bahiagrass. Fla. Coop. Ext. Serv., IFAS, Univ. of Florida, Circular 321B.

Chao, T. T., M. E. Harward, and S. C. Fang. 1962. Movement of S^{35} tagged sulfate through soil columns. Soil Sci. Soc. Am. Proc. 26:27-32.

Cheng, B. T., and G. J. Ouellette. 1973. Molybdenum as a plant nutrient. Soils Fert. 36:207-215.

Chesnin, L., and C. H. Yien. 1950. Turbidimetric determination of available sulfates. Soil Sci. Soc. Am. Proc. 15:149-151.

Coleman, N. T., E. J. Kamprath, and S. B. Weeb. 1958. Liming. Adv. Agron. 10:475-523.

Coleman, R. 1966. The importance of sulfur as a plant nutrient in world crop production. Soil Sci. 101:230-239.

Cowling, D. W., and L. H. P. Jones. 1971. Sulphur deficiency of two forage plants in England. The Sulphur Inst. J. 6(4):8-13.

Cram, J. 1983. Characteristics of sulfate transport accross plasmalemma and tonoplast. Plant Physiol. 72:204-211.

Daigger, L. A., and R. L. Fox. 1971. Nitrogen and sulfur nutrition of sweet corn in relation to fertilization and water composition. Agron. J. 63:729-730.

Dawson, M. D. 1969. Sulphur on pasture legumes in Oregon. The Sulphur Inst. J. 5(3):16-18.

Dijkshoorn, W., J. E. M. Lampe, and P. F. J. Van Burg. 1960. A method of diagnosing the sulfur nutrition status of herbage. Plant Soil 13:227-241.

Dijkshoorn, W., and A. L. Van Wijk. 1967. The sulphur requirements of plants as evidenced by the sulphur-nitrogen ratio in the organic matter. A review of published data. Plant Soil. 26:129-157.

Drlica, D. M., and T. L. Jackson. 1979. Effects of stage of maturity on P and S critical levels in subterranean clover. Agron. J. 71:824-828.

Epstein, E. 1972. Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, Inc., New York.

Fales, S., and L. Thompson. 1982. Review of literature. p. 3-6. In J. J. Mortvedt, and O. E. Anderson (ed.) Forage legumes: diagnosis and correction of molybdenum and manganese problems. Georgia Agric. Exp. Stn Bull 278.

Fernandez, L. R. 1983. Effects of amendments incorporated into the surfaces of a Florida Entisol and an Ultisol on subsoil properties and corn (Zea mays L.) responses. M.S. thesis. Univ. of Florida, Gainesville.

Fox, R. L., M. Asghar, W. J. Cable. 1983. Sulfate accretions in soils of the tropics. p. 39-53. In G. J. Blair and A. R. Till (ed.) Sulfur in south-east Asian and South Pacific agriculture. University of New England, Armidale, Australia.

Fox, R. L., B. T. Kang, and D. Nangju. 1977. Sulfur requirements of cowpea and implications for production in the tropics. Agron. J. 69:201-205.

Fox, R. L., R. A. Olson, and H. F. Rhoades. 1964. Evaluating the sulfur status of soils by plant and soil tests. Soil Sci. Soc. Am. Proc. 28:243-246.

Freney, J. R. 1958. Determination of water-soluble sulphate in soils. Soil Sci. 86:241-244.

Freney, J. R. 1961. Some observations on the nature of organic sulphur compounds in soils. Aust. J. Agric. Res. 21:424-432.

Freney, J. R. 1967. Sulfur-containing organics. p. 220-259. In A. D. McLaren and G. H. Peterson (ed.) Soil biochemistry. Marcel Dekker, Inc., New York.

Freney, J. R., G. E. Melville, and C. H. Williams. 1975. Soil organic matter fractions as sources of plant available sulphur. Soil Biol. Biochem. 7:217-221.

Freney, J. R., and K. Spencer. 1960. Soil sulphate changes in the presence and absence of growing plants. Aust. J. Agric. Res. 11:339-345.

Freney, J. R., and F. J. Stevenson. 1966. Organic sulfur transformations in soils. Soil Sci. 101:307-316.

Freund, R. J., and R. C. Littell. 1981. SAS for linear models -- a guide to the Anova and GLM procedures. SAS series in statistical applications. SAS Institute Inc., Cary, NC.

Gallaher, R. N., and F. R. Arias. 1985. Survey of sulfur deficiency in maize. Agron. Abstr. American Society of Agronomy, Madison, WI. p. 172.

Gast, R. G. 1977. Surface and colloid chemistry. p. 27-73. In J. B. Dixon, and S. B. Weed (ed.) Minerals in soil environments. Soil Science Society of America, Madison, WI.

Gates, C. T. 1974. Nodule and plant development in Stylosanthes humilis H. B. K.: symbiotic response to phosphorus and sulphur. Aust. J. Bot. 22:45-55.

Goldberg, E. D. 1963. The ocean as a chemical system. p. 3-25 In M. N. Hill (ed.) The sea. Vol. 2. Interscience Publishers, John Wiley and Sons, New York.

Grava, J. 1971. The sulphur picture in Minnesota. The Sulphur Inst. J. 7(1):12-14.

Gupta, U. C., and J. Lipsett. 1981. Molybdenum in soils, plants, and animals. Adv. Agron. 34:73-115.

Gupta, U. C., and D. C. Munro. 1969. Influence of sulfur, molybdenum and phosphorus on chemical composition and yields of Brussels sprouts and of molybdenum on sulfur contents of several plant species grown in the greenhouse. Soil Sci. 107:114-118.

Harris, H. C., V. N. Schroder, and R. L. Gilman. 1968. Nutrient deficiency effects on yield and chemical composition of plants grown on Leon fine sand. Fla. Agric. Exp. Stn. Tech. Bull. 725.

Harward, M. E., T. T. Chao, and S. C. Fang. 1962. The sulfur status and sulfur supplying power of Oregon soils. Agron. J. 54:101-106.

Hawes, R. L., J. L. Sims, and K. L. Wells. 1976. Molybdenum concentration of certain crop species as influenced by previous applications of molybdenum fertilizer. Agron. J. 68:217-218.

Hern, J. L. 1984. Determination of total sulfur in plant materials using an automated sulfur analyzer. Commun. Soil Sci. Plant Anal. 15:99-107.

Hingston, F. J., A. M. Posner, and J. P. Quirk. 1972. Anion adsorption by goethite and gibbsite. I. The role of the proton in determining adsorption envelopes. J. Soil Sci. 23:177-192.

Hinman, W. C. 1964. Fixed ammonium in some Saskatchewan soils. Can. J. Soil Sci. 44:151-157.

Hue, N. V., F. Adams, and C. E. Evans. 1984. Plant-available sulfur as measured by soil-solution sulfate and phosphate-extractable sulfate in a Ultisol. Agron. J. 76:726-730.

Impithuksa, V., and W. G. Blue. 1977. The fate of fertilizer nitrogen applied to Pensacola bahiagrass on sandy soils as indicated by nitrogen-15. Proc. Soil Crop Sci. Soc. Fla. 37:213-217.

Johnson, C. M. 1966. Molybdenum. p. 286-301. In H. D. Chapman (ed.) Diagnostic criteria for plant and soils. Univ. California, Division of Agricultural Sciences, Riverside, CA.

Johnson, C. M., and H. Nishita. 1952. Microestimation of sulfur in plant materials, soils, and irrigation waters. Anal. Chem. 24:736-742.

Jones, G. B., and G. B. Belling. 1967. The movement of copper, molybdenum, and selenium in soils as indicated by radioctive isotopes. Aust. J. Agric. Res. 18:733-740.

Jones, M. B. 1962. Total sulfur and sulfate content in subterranean clover as related to sulfur responses. Soil Sci. Soc. Am. Proc. 26:482-484.

Jones, M. B. 1963. Effect of sulfur applied and date of harvest on yield, sulfate sulfur concentration, and total sulfur uptake of five annual grassland species. Agron. J. 55:251-254.

Jones, M. B. 1964. Effect of applied sulfur on yield and sulfur uptake of various California dryland pasture species. Agron. J. 56:235-237.

Jones, M. B., and W. E. Martin. 1964. Sulphate-sulfur concentration as an indicator of sulfur status in various California dryland pasture species. Soil Sci. Soc. Am. Proc. 28:539-541.

Jones, R. K., M. E. Probert, and B. J. Crack. 1975. The occurrence of sulphur deficiency in the Australian tropics. p. 127-136. In McLachlan, K. D. (ed.) Sulphur in Australasian agriculture. Sydney University Press, Australia.

Jordan, H. V., and C. E. Bardsley. 1958. Response of crops to sulfur on Southeastern soils. Soil Sci. Soc. Proc. 22:254-256.

Jordan, H. V., R. W. Pearson, and C. E. Bardsley. 1961. Sulfur as a plant nutrient in soils of the Southeastern United States. Trans. Int. Congr. Soil Sci., 7th. 4:563-572.

Kamprath, E. J., W. L. Nelson, and J. W. Fitts. 1956. Effect of pH, sulfate, and phosphate concentrations on the adsorption of sulfate by soils. Soil Sci. Soc. Am. Proc. 20:463-466.

Kamprath, E. J., and A. R. Till. 1983. Sulfur cycling in the tropics. p. 1-14. In G. J. Blair and A. R. Till (ed.) Sulfur in south-east Asian and south Pacific agriculture. University of New England, Armidale, Australia.

Karimian, N., and F. R. Cox. 1978. Adsorption and extractability of molybdenum in relation to some chemical properties of soil. Soil Sci. Soc. Am. J. 42:757-761.

Khan, S. U., R. O. Cloutier, and M. Hidiroglou. 1979. Atomic absorption spectroscopic determination of molybdenum in plant tissue and blood plasma. J. Assoc. Off. Anal. Chem. 62:1062-1064.

Koger, M., W. G. Blue, G. B. Killinger, R. E. L. Greene, H. C. Harris, J. M. Myers, A. C. Warnick, and N. Gammon, Jr. 1961. Beef production returns from eigth pasture programs in north central Florida. Fla. Agric. Exp. Stn. Bull. 631.

Koger, M., W. G. Blue, G. B. Killinger, R. E. L. Greene, J. M. Myers, N. Gammon, Jr., A. C. Warnick, and J. R. Crockett. 1970. Production response and economic returns from five pasture programs in North Central Florida. Fla. Agric. Exp. Stn. Bull. 740.

Koger, M., R. E. L. Greene, G. B. Killinger, W. G. Blue, and J. M. Myers. 1977. Pasture programs and beef cattle breeding systems for beef production in north central Florida. Fla. Agric. Exp. Stn. Bull. 789.

Lauenroth, W. K., and E. M. Preston. 1984. The effects of SO_2 on a grassland- a case study in the Northern Great Plains of the United States. Springer-Verlag New York Inc., New York.

Leco Corporation. 1980. Leco Instruction manual 200-170. Saint Joseph, MI.

Leffel, R. C., and P. B. Gibson. 1973. White clover. p. 167-176. In M. E. Heath, D. S. Metcalfe, and R. F. Barnes (ed.) Forages, the science of grassland agriculture. 3rd ed. The Iowa State University Press, Ames, IA.

Longnecker, T. C., and H. B. Sprague. 1940. Rate of penetration of lime in soils under permanent grass. Soil Sci. 50: 277-288.

Lowe, L. E., and W. A. De Long. 1963. Carbon bonded sulfur in selected Quebec soils. Can. J. Soil Sci. 43:151-155.

Lucas, R. E., and B. D. Knezec. 1972. Climatic and soil conditions promoting micronutrient deficiencies in plants. p. 265-288. In J. J. Mortvedt, P. M. Giordano and W. L. Lindsay (ed.) Micronutrients in agriculture. Soil Science Society of America, Madison, WI.

Lutz, J. A., and G. D. Jones. 1974. Effects of deep placement of limestone and fertility treatments on the chemical composition of soybeans and on certain properties of a Tatum soil. Comm. Soil Sci. Plant Anal. 5:399-441.

Martin, W. E. and J. E. Matocha. 1973. Plant analysis as an aid in the fertilization of forage crops. p. 393-426. In L. M. Walsh and J. D. Beaton (ed.) Soil testing and plant analysis. Am. Soc. of Agron., Madison, WI.

Massuomi, A., and A. H. Cornfield. 1963. A rapid method for determining sulphate in water extracts of soil. Analyst. 88:321-322.

McClung, A. C., L. M. M. Freitas, and W. L. Lott. 1959. Analyses of several Brazilian soils in relation to plant responses to sulfur. Soil Sci. Soc. Am. Proc. 23:221-224.

McKell, C. M., and W. A. Williams. 1960. A lysimeter study of sulfur fertilization of an annual-range soil. J. Range Manage. 13:113-117.

McLaughlin, B. D., and I. C. R. Holford. 1982. Initial and medium-term responses of white clover to three sulfur fertilizers on a basaltic soil. Aust. J. Exp. Agric. Anim. Husb. 22:95-99.

McNaught, K. J. 1970. Diagnosis of mineral deficiencies in grass-legume pastures by plant analysis. p. 334-338. In Proc. Int. Grassl. Congr., 11th, Surfers Paradise, Queensland, Australia.

McNaught, K. J., and C. During. 1970. Relations between nutrient concentrations in plant tissues and responses of white clover to fertilizers on a gley podzol near Westport. N. Z. J. Agric. Res. 13:567-590.

Metson, A. J. 1973. Sulphur in forage crops. Tech. Bull. No. 20. The Sulphur Institute, Washington, D. C.

Mitchell, C. C., Jr. 1980. The sulfur fertility status of Florida soils. Ph.D. diss. Univ. of Florida, Gainesville (Diss. Abstr. 42:456B).

Mitchell, C. C., Jr., and W. G. Blue. 1981. The sulfur fertility status of Florida soils. I. Sulfur distribution in Spodosols, Entisols, and Ultisols. Proc. Soil Crop Sci. Soc. Fla. 40:71-76.

Neller, J. R. 1952. Sulfur in relation to fertilizers and soil amendments for grasslands. Proc. Int. Grassl. Congr., 6th 728-734.

Neller, J. R. 1958. Influence of fertilizers containing superphosphate on the soluble sulfate and phosphate of Blanton fine sand. Proc. Soil Crop Sci. Soc. Fla. 18:193-196.

Neller, J. R., and F. D. Bartlett. 1959. Growth of forage crops on Leon fine sand as affected by adding sulfur to rock phosphate. Soil Sci. 88:331-335.

Neller, J. R., D. W. Jones, N. Gammon, Jr., and R. B. Forbes. 1951a. Leaching of fertilizer phosphorus in acid sandy soils as affected by lime. Fla. Agric. Exp. Stn. Circular S-32.

Neller, J. R., G. B. Killinger, D. W. Jones, R. W. Bledsoe, and H. W. Lundy. 1951b. Fertilizer should contain a source of sulfur for clover pastures in many areas of Florida. Fla. Agric. Exp. Stn. Circular S-35.

Nelson, D. W., and L. E. Sommers. 1972. A simple digestion procedure for estimation of total nitrogen in soils and sediments. J. Environ. Qual. 1:423-425.

Nelson, W. L., A. Mehlich, and E. Winters. 1953. The development, evaluation, and use of soil tests for phosphorus availability. In W. H. Pierre and A. G. Norman (ed.) Soil and fertilizer phosphorus. Agronomy 4:153-188. Am. Soc. of Agron., Madison, WI.

Nuttal, W. F. 1985. Effect of N, P, and S fertilizers on alfalfa grown on three soil types in northeastern Saskatchewan. II. Nitrogen, P, and S uptake and concentration in herbage. Agron. J. 77:224-228.

Ozanne, P. G. 1980. Phosphate nutrition of plants - a general treatise. p. 559-589. In F. E. Khasawneh, E. C. Sample, and E. J. Kamprath (ed.) The role of phosphorus in agriculture. Am. Soc. Agr., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Pavan, M. A., F. T. Bingham, and P. F. Pratt. 1984. Redistribution of exchangeable calcium, magnesium, and aluminum following lime or gypsum applications to a Brazilian Oxisol. Soil Sci. Soc. Am. J. 48:33-38.

Peaslee, D. E. 1982. Introduction. p. 2-3. In J. J. Mortvedt and O. E. Anderson (ed.) Forage legumes: diagnosis and correction of molybdenum and manganese problems. Georgia Agric. Exp. Stn. Bull. 278.

Prine, G. M., O. C. Ruelke, C. E. Dean, and D. D. Baltensperger. 1984. Performance of winter forage legumes at Gainesville, Florida over eight growing seasons 1975-76 to 1982-83. Dept. of Agron. Agric. Exp. Stn. Agronomy Research Report AY 84-10.

Probert, M. E., and S. S. R. Samosir. 1983. Sulfur in non-flooded tropical soils. p. 15-27. In G. J. Blair and A. R. Till (ed.) Sulfur in south-east Asian and south Pacific agriculture. University of New England, Armidale, Australia.

Randall, P. J., and H. Sakai. 1983. Sulfur analytical methods for soils, plants, and animal samples. p. 255-269. In G.J. Blair and A. R. Till (ed.) Sulfur in south-east Asian and south Pacific agriculture. University of New England, Armidale, Australia.

Rasmussen, P. E., R. E. Ramig, L. G. Ekin, and C. R. Rohde. 1977. Tissue analyses guidelines for diagnosing sulfur deficiency in white wheat. Plant Soil. 46:153-163.

Reddy, G. D., A. M. Alston, and K. G. Tiller. 1981. Effects of fertilizer on concentrations of copper, molybdenum, and sulfur in subterranean clover (Trifolium subterraneum). Aust. J. Exp. Agric. Anim. Husb. 21:491-497.

Reeve, N. G., and M. E. Sumner. 1972. Amelioration of subsoil acidity in Natal Oxisols by leaching of surface-applied amendments. Agrochemophysica 4:1-6.

Rehm, G. W., and A. C. Caldwell. 1968. Sulfur supplying capacity of soils and the relationship to soil type. Soil Sci. 105:355-361.

Reisenauer, H. M. 1963. The effect of sulfur on the absorption and utilization by peas. Soil Sci. Soc. Am. Proc. 27:553-555.

Reisenauer, H. M., L. M. Walsh, and R. G. Hoeft. 1973. Testing soils for sulphur, boron, molybdenum, and chlorine. p. 173-200. In L. M. Walsh, and J. D. Beaton (ed.) Soil testing and plant analysis. Soil Sci. Soc. Am., Inc., Madison, WI.

Rennenberg, H. 1984. The fate of excess sulfur in higher plants. Ann. Rev. Plant Physiol. 35:121-153.

Rhue, R. D., and G. Kidder. 1983. Procedures used by the IFAS Extension Soil Testing Laboratory and interpretation of results. Florida Cooperative Extension Service, IFAS, Univ. of Florida, Gainesville, Fl., Circular 596.

Robinson, P. J., and R. K. Jones. 1972. The effect of phosphorus and sulphur fertilization on the growth and distribution of dry matter, nitrogen, phosphorus, and sulphur in Townsville stylo (Stylosanthes humilis). Aust. J. Agric. Res. 23:633-640.

Robson, A. D. 1978. Mineral nutrients limiting nitrogen fixation in legumes. p. 277-293. In C. S. Andrew, and E. J. Kamprath (ed.) Mineral nutrition of legumes in tropical and subtropical soils. CSIRO, Melbourne, Australia.

Salisbury, F. B., and C. W. Ross. 1978. Plant physiology. 2nd ed. Wadsworth Publishing Co., Belmont, CA.

Sanchez, P. A., and G. Uehara. 1980. Management considerations for acid soils with high phosphorus fixation capacity. p. 471-514. In F. E. Khasawneh, E. C. Sample, and E. J. Kamprath (ed.) The role of phosphorus in agriculture. Am. Soc. Agr., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

SAS. 1982. SAS User's guide: basics, 1982 ed. SAS Institute Inc., Cary, NC.

Shock, C. C., M. B. Jones, W. A. Williams, and D. M. Center. 1983. Effect of sulfur fertilization on three annual range species. I. Laughlin soil experiment. Agron. J.-75:515-520.

Shock, C. C., M. B. Jones, W. A. Williams, and D. M. Center. 1984a. Competition for S and N by associations of three annual range species in lysimeters. Plant Soil. 81:311-321.

Shock, C. C., W. A. Williams, M. B. Jones, D. M. Center, and D. A. Phillips. 1984b. Nitrogen fixation by subclover associations fertilized with sulfur. Plant Soil. 81: 323-332.

Siebert, B. D., and P. Vijchulata. 1983. Sulfur in animal nutrition. p. 87-96. In G. J. Blair and A. R. Till (ed.) Sulfur in south-east Asian and south Pacific agriculture. University of New England, Armidale, Australia.

Singh, M., and V. Kumar. 1979. Sulfur, phosphorus and molybdenum interactions on the concentration and uptake of molybdenum in soybean plants (Glycine max). Soil Sci. 127:307-312.

Smith, P. F., G. K. Rasmussen, and G. Hrnciar. 1962. Leaching studies with metal sulfates in light sandy citrus soil in Florida. Soil Sci. 94:235-238.

Spencer, K. 1959. Growth and chemical composition of white clover as affected by sulphur supply. Aust. J. Agric. Res. 10:500-509.

Spencer, K. 1975. Sulphur requirements of plants. p. 98-108. In K. E. McLachlan (ed.) Sulphur in Australasian Agriculture, Sydney University Press, Sydney, Australia.

Spencer, K. 1978. Sulphur nutrition of clover: Effects of plant age on the composition-yield relationship. Commun. Soil Sci. Plant Anal. 9:883-895.

Spencer, K., and J. R. Freney. 1980. Assessing the sulfur status of field-grown wheat by plant analysis. Agron. J. 72:469-472.

Spencer, K., J. R. Freney, and M. B. Jones. 1977a. Diagnosis of sulphur deficiency in subterranean clover. Sulphur in Agric. 1:12-15.

Spencer, K., M. B. Jones, and J. R. Freney. 1977b. Diagnostic indices for sulphur status of subterranean clover. Aust. J. Agric. Res. 28:401-412.

Stout, P. R., W. R. Meagher, G. A. Pearson, and C. M. Johnson. 1951. Molybdenum nutrition of crop plants. I- The influence of phosphate and sulfate on the absorption of molybdenum from soils and solution cultures. Plant and soil 3:51-87.

Stevenson, F. J. 1982. Humus chemistry. John Wiley and Sons, New York.

Stevenson, F. J., and M. S. Ardakani. 1972. Organic matter reactions involving micronutrients in soils. p. 79-114. In J. J. Mortvedt, P. M. Giordano, and W. L. Lindsay (ed.) Micronutrients in agriculture. Soil Sci. Soc. Am., Madison, WI.

Stewart, B. A. 1969. N:S ratios. A guideline to sulphur needs. The Sulphur Inst. J. 5(3):12-15.

Sulphur Institute. 1982. The fourth major nutrient. The Sulphur Institute. Washington, D.C.

Tabatabai, M. A. 1982. Sulfur. In A. L. Page (ed.) Methods of soil analysis. Part 2 - Chemical and microbiological properties. Agronomy 9:501-538. Am. Soc. Agron., and Soil Sci. Soc. Am., Madison, WI.

Tabatabai, M. A., and J. M. Bremner. 1970a. An alkaline oxidation method for determination of total sulfur in soils. Soil Sci. Soc. Am. Proc. 34:62-65.

Tabatabai, M. A., and J. M. Bremner. 1970b. Comparison of some methods for determination of total sulfur in soils. Soil Sci. Soc. Am. Proc. 34:417-420.

Tabatabai, M. A., and J. M. Bremner. 1972. Forms of sulfur, and carbon, nitrogen, and sulfur relationships, in Iowa soils. Soil Sci. 114:380-386.

Tandon, H. L. S. 1984. Sulphur research and agricultural production in India. Fertilizer Development and Consultation Organisation, New Delhi, India.

Tengku Maamun, T. A. M. 1985. Effects of micronutrients and sulfur on white clover performance and nutrient concentrations in Myakka fine sand (Aeric Haplaquod). M. S. thesis. Univ. of Florida, Gainesville.

Terman, G. L., S. E. Allen, and P. M. Giordano. 1973. Dry matter yield - N and S concentration relationships and ratios in young corn plants. Agron. J. 65:633-636.

Thompson, C. J., and C. S. Allbright. 1970. Total sulfur. p. 89-144. In J. H. Karchmer (ed.) The analytical chemistry of sulfur and its compounds. Part 1. John Wiley and Sons, New York.

Thompson, J. F. 1967. Sulfur metabolism in plants. Ann. Rev. Plant Physiol. 18:59-84.

Thompson, L. G., Jr., and J. R. Neller. 1963. Sulfur fertilization of winter clovers, coastal bermudagrass, and corn on North and West Florida soils. Fla. Agric. Exp. Stn. Bull. 656.

Tisdale, S. L., W. L. Nelson, and J. D. Beaton. 1985. Soil fertility and fertilizers. 4th ed. Macmillan Publishing Co., New York.

Ulrich, A., and L. O. Hylton, Jr. 1968. Sulfur nutrition of Italian ryegrass measured by growth and mineral content. Plant Soil 29:274-284.

Walker, D. R., and C. F. Bentley. 1961. Sulphur fractions of legumes as indicators of sulphur deficiency. Can. J. Soil Sci. 41:164-168.

Walker, D. R., and G. Doornenbal. 1972. Soil sulfate. II. As an index of the sulfur available to legumes. Can. J. Soil Sci. 52:261-266.

Walker, T. W., A. F. R. Adams, and H. D. Orchiston. 1956. The effect of levels of calcium sulphate on the yield and composition of a grass and clover pasture. Plant Soil 7:290-300.

Walkley, A. 1947. A critical examination of a rapid method for determining organic carbon in soils. Soil Sci. 63:251-264.

Ward, G. M. 1978. Molybdenum toxicity and hypocuprosis in ruminants: a review. J. Anim. Sci. 46:1078-1085.

Ward, C. Y., and V. H. Watson. 1973. Bahiagrass and carpet grass. p. 314-320. In M. E. Heath, D. S. Metcalfe, and R. F. Barnes (ed.) Forages, the science of grassland agriculture. 3rd ed. Iowa State University Press, Ames, IA.

Watson, E. R. 1969. The influence of subterranean clover pastures on soil fertility. III. The effect of applied phosphorus and sulphur. Aust. J. Agric. Res. 20:447-456.

Whitehead, D. C. 1964. Soil and plant-nutrition aspects of the sulphur cycle. Soil Fert. 27:1-8.

Williams, W. A., C. M. McKell, and J. N. Reppert. 1964. Sulfur fertilization of an annual-range soil during years of below-normal rainfall. J. Range Manage. 17:1-5.

Williams, C. H., and A. Steinbergs. 1959. Soil sulphur fractions as chemical indices of available sulphur in some Australian soils. Aust. J. Agric. Res. 10:340-352.

Woodhouse, W. W., Jr. 1969. Long-term fertility requirements of coastal Bermudagrass. III. Sulphur. Agron. J. 61:705-708.

Yuan, T. L. 1963. Some relationships among hydrogen, aluminum, and pH in solution and soil systems. Soil Sci. 95:155-163.

BIOGRAPHICAL SKETCH

Francisco Antonio Monteiro was born on 03 April 1949, in Rio das Pedras, State of Sao Paulo, Brazil. He is the son of Joao Monteiro and Margarida Negresolo Monteiro. He married Miss Marisa Martins in 1976, and they have one daughter, Juliana.

He completed elementary, middle, and high school at the Instituto de Educaao "Sud Mennucci" in Piracicaba, State of Sao Paulo. He received his B.S. and M.S. degrees from the Escola Superior de Agricultura "Luiz de Queiroz", Universidade de Sao Paulo, in Piracicaba. In 1972 he was awarded the degree of Engenheiro Agronomo, and in 1981 he received the Master of Science degree in soils and plant nutrition.

From December 1972 to January 1974 he worked for Proagri-Projetos, Planejamentos e Pesquisas. In January 1974, he joined the staff of the Instituto de Zootecnia, Secretaria de Agricultura do Estado de Sao Paulo, to work on forage fertilization.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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